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Symposium on the Physiology of Cardiac Muscle

Introduction

MODERN study of the physiology of cardiac muscle began with the discovery of the circulation by Harvey in 1620 [*1*]. The conclusions which Harvey drew from his gross study of the motion of the heart and arteries have undergone remarkably little modification in the intervening three and a half centuries. Today, it is the molecular activity in cardiac muscle which is responsible for the motion of the heart that intrigues us. Although much remains to be learned, there is little doubt that the processes responsible for the heart beat are carefully organized biochemical reactions which culminate in mechanical work.

The advances which have led to our current knowledge of cardiac function and metabolism have been due as much to the introduction of new technics as to the intuitive elaboration of new concepts. Although the discovery of the stethoscope by Laennec in 1816 provided a new instrument for the clinical appraisal of cardiac function and the categorization of certain cardiac disorders, the most significant advances have been made since 1900. The invention of the electrocardiograph by Einthoven [*2*], development of polygraphic methods for recording pulse waves by Lewis [*3*], and the study of the isolated perfused heart by Starling [*4*] led to basic concepts regarding the functional adaptability and bioelectric behavior of the myocardium. Study of the electrical properties of cardiac muscle has been extended in the clinical direction to provide a more satisfying view of the course of epicardial depolarization through the vector cardiogram, and in the fundamental direction through the study of the action potentials of single cardiac fibers. The biochemical link between membrane depolarization and the initiation of contraction remains a mystery. One essay in this symposium deals specifically with advances in the field of electrophysiology of the heart. Starling's law of the heart, developed through a study of the work performance of the isolated heart under varying conditions of venous supply and peripheral resistance, is still being scrutinized for applicability to various animal preparations, including intact man, and this subject is considered in another paper in this symposium.

When Forssmann [*5*] inserted a catheter through the brachial vein into his own heart, a new era of cardiac study was ushered in. The technics of cardiac catheterization were then established by Cournand and his colleagues [*6*], and the information obtained by direct measurement of intracardiac pressures and flows aided materially in the advancement of our knowledge of cardiac physiology. New insights were obtained regarding the hemodynamic changes in congenital and acquired valvular heart disease. The initially fortuitous intubation of the coronary sinus in the course of cardiac catheterization opened up a new frontier for the study of myocardial metabolism in animals [*7*] and in man [*8,9*]. By simultaneous measurements [*10*] of coronary flow through adaptation of Kety's method [*11*] for measuring cerebral blood flow, it was possible to measure the rate of respiratory gas exchange and uptake of substrate by intact myocardial muscle under a variety of conditions [*12–15*]. The advent of open heart surgery with its extracorporeal perfusion technics has provided even more accessibility to the intact myocardium in the living organism. This

succession of technical developments in the study of the heart and circulation has stimulated a great deal of research and provided evidence for some new concepts about the over-all function, metabolism and work of the intact heart in health and in disease. Study of the intact heart is necessarily limited to measurement of changes in the concentration of substrates and ions in the perfusing blood, and although such studies yield informative data on the net rate of substrate utilization and oxidation by the heart *in situ*, very little about the intermediary metabolism of the organs can be learned from such preparations.

It is obvious that a multifaceted approach to the study of the myocardium is essential for a complete understanding of its function. Such studies require a further dissection of the heart into its component cells, subcellular particulates (mitochondria, microsomes, nuclei and membranes), and enzyme systems. Information gained from these various preparations will, of necessity, be different—some of it reflecting the potentiality of the organ, some of it the actual performance. It is obvious that as one goes from examination of the intact organ through the gamut of the perfused organ, the surviving tissue slice, the whole homogenate, the fractionated homogenate, the particulate suspension, and ultimately to the purified enzyme system, one goes from the highly organized and most complex, most physiologic, and least interpretable behavior to the least complex, most interpretable, but least physiologic behavior. A synthesis of data obtained from these various preparations is required for complete information about cardiac physiology, and in the papers presented in this symposium practically all the technics cited will be mentioned in relationship to the problem.

In addition to this organocentric evolution of methodology for study of cardiac physiology, important contributions have been made in the past by physiologists concerned with the study of the contractility of striated muscle. The fusion of these two streams of research has brought us to our current position. The biochemical study of muscular contraction began with the discovery of myosin by Kühne in 1859 [*16*]. Kühne noted that saline extracts of skeletal muscle produced gels *in vitro* and speculated about the relationship of this gel formation to contraction. Subsequent research dealing with the purification and characterization of mammalian skeletal myosin by Edsall, Weber, Bailey, Szent-Györgyi and Mommaerts [*17*] led to estimation of its size, shape and molecular weight. Szent-Györgyi's discovery of actin in 1943 [*18*] made possible the reconstruction of actomyosin systems which manifested *in vitro* contractility. It is noteworthy however, that throughout this period of intensive study of skeletal muscle comparatively little attention was given to cardiac muscle as a source of the contractile proteins. Recent studies of the contractile proteins in cardiac muscle suggest strongly that these proteins of the heart are related to, but not identical with, those from skeletal muscle. It seems likely that whole families of actins and myosins may be present in various contractile tissues, and the study of their precise interrelationships constitutes an interesting problem for future investigation.

As regards the thermodynamics of muscular contraction, mention should be made of the brilliant researches of Meyerhof [*19*], whose studies of glycolysis stemmed from a curiosity about the mechanism of formation of lactic acid in skeletal muscle during contraction; of the incisive studies of Lundsgaard [*20*] who showed paradoxically that lactic acid formation was not essential for contraction, and of the classic studies of Hill [*21*] who studied the relationship between tension, velocity of shortening, and heat production in the frog sartorius muscle. In fact, Huxley [*22*] has shown that the newly proposed "sliding model" of interdigitating actin and myosin filaments, satisfies Hill's force-velocity equation, and these correlations are discussed extensively in this symposium.

The pathogenesis of congestive heart failure remains a problem. It seems from the biochemical point of view that heart failure is a disorder of multiple etiology. Studies seeking to clarify the pathologic physiology of congestive heart failure have led to the conclusion that, in general, high output failure represents metabolic heart disease in which the whole organism participates, and low output failure represents non-metabolic heart disease in which only the contractile mechanism in the heart is defective. The presently controversial views on the precise location of the biochemical lesions in these syndromes is discussed at length in the papers which follow.

One can hardly speculate about the biochemistry of the failing heart without thinking of digitalis. It is of interest that the cardiotonic drugs have been used as pharmacologic agents in man for at least 2,000 years. Withering introduced digitalis leaf to the practice of medicine nearly 200 years ago, and the foxglove and its

purified glycosides are still used empirically in the treatment of congestive heart failure by physicians. Despite an enormous amount of research, which has been carried out over the past fifty years, we know little more than the chemical structure of the cardiac glycosides and that these intensely potent agents cause a variety of biochemical effects in tissues, including alteration in membrane potential, ionic shifts, alteration in respiration, and changes in the behavior of the contractile proteins [*23*]. Out of the welter of data it is not clear what effect, if any, which has been observed to date is responsible for the inotropic effect of the drug in the failing heart. It seems unlikely that the effect is on the pathways of energy production (glycolysis, Krebs cycle oxidations, electron transport or oxidative phosphorylation) since these are common to all tissues. To attain the inotropic effect, it would seem more likely that the digitalis glycosides influence some reaction unique to cardiac muscle which is more prominent in the failing heart.

If one attempts to frame a hypothesis at the molecular level, one is balked by the extremely low concentrations of digitoxin which are effective in man, i.e., *ca* 60 μg. per kg. of heart muscle. This is a molar ratio of digitoxin to total heart protein of about 10^{-4}. To postulate that digitoxin acts to modify permeability of a membrane or the contractility of a protein, one must assume that digitoxin combines with *some* protein in a molar ratio of 1:0. This means, in effect, that the protein in question must be in a relatively low concentration (at a molecular weight of 200,000, about 2 mg. per cent). If this protein were an enzyme, and the behavior of this enzyme were radically altered by cardiac glycoside, uptake of the drug would change the rate of delivery of product. If the product, in turn, influenced the contractility of the myofibril, the necessary amplification to account for the pharmacologic activity of digitalis would be provided.

$$(1) \qquad E + dig \rightleftarrows E\text{-}dig$$

$$(2) \qquad R \xrightarrow{E\text{-}dig} P$$

$$(3) \qquad AM_1 \xrightarrow{P} AM_2$$

where E = enzyme, dig = digitoxin, R = reactant, P = product, AM_1 = failing actomyosin, and AM_2 = altered actomyosin.

The permeability hypothesis which postulates that digitalis acts by altering the entry of a given ion which, in turn, influences the contractility of the myofibril would meet the requirements of this analysis. Likewise, a hypothesis which provides for fixation of digitoxin in combination with a myofibrillar protein of low concentration, which, in turn, could modify the behavior of that filament or its constituent contractile proteins would suffice. Much remains to be learned.

It is obvious from the progress which has been made in understanding the physiology of cardiac muscle thus far that contributions from all the relevant sciences in the study of this problem are essential. It appears to me that although contributions by clinicians and physiologists have been important in the past, the area for study now is at the molecular level, and additional biochemists and biophysicists should be enlisted to study the specific biochemistry of the normal and failing heart, the normal and abnormal contractile process, and the mechanism of action of the cardiotonic drugs.

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Structure of the Cardiac Muscle Cell*

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IN 1664, the muscular nature of the heart was proclaimed in a monograph by Stensen [*1*]. With the advent of the light microscope, histologic details were adduced to confirm the structural resemblance of heart muscle to skeletal muscle. By 1932 Cohn [*2*] was able to portray heart muscle as differing from skeletal muscle in only two particulars: (1) a "syncytial arrangement"; and (2) the presence of a "characteristic structure, the intercalated disc." Early attempts at evaluation of the substructure of muscle were limited by inadequate tissue preparatory technics; within the last decade, however, improved tissue processing has enabled electron microscopists to make significant observations concerning the ultrastructural components of striated muscle, including cardiac muscle. This presentation will describe and illustrate these components and will discuss some of the intriguing interpretations that have been offered concerning the functional significance of the ultrastructural details.

MATERIAL AND METHODS

Papillary muscles of rat and dog hearts were fixed for twenty to thirty minutes, under slight tension, with a buffered 2 per cent osmium tetroxide solution. Selected muscle segments were further fixed at 4°C. for two to six hours. Acetone dehydration was followed by embedding in methacrylate or araldite. All tissue was exposed for one hour to 1 per cent phosphotungstic acid during the final phase of dehydration. Sectioning was accomplished with a diamond knife in a Porter-Blum microtome. Some sections were stained with potassium permanganate [*3*] or uranyl nitrate. Electron micrographs were made with an RCA-EMU-3B or a Siemens Elmiskop I.

GENERAL CYTOLOGY

The papillary muscles of rat and dog hearts consist predominantly of myocardial fibres. The intercellular space varies in width. Capillaries are frequently encountered. Occasional fibroblasts are found, but collagen is scanty. (Fig. 1.) Nerves are not observed; however, tissue immediately adjacent to the endocardium was not examined. Fawcett and Selby [*4*] found numerous unmyelinated nerves beneath the endocardium of the turtle atrium.

SARCOLEMMA

In heart muscle the surface membrane usually appears as a thin dense line, forming the cell boundary. (Figs. 1, 2 and 3.) Occasionally, with osmium tetroxide fixation, the sarcolemma demonstrates a "unit membrane" structure, as defined by Robertson [*5*]. On the external aspect the surface membrane is invested by a moderately dense basement membrane of rather uniform width. (Figs. 1 and 3.) Frequently pinocytotic vesicles of the type described by Lewis [*6*] extend from the cell surface into the adjacent cytoplasm. (Figs. 1 and 3.) Segments of the sarcoplasmic reticulum frequently come into close relationship with the sarcolemma (Figs. 1 and 3); however, actual continuity of the sarcolemma with the sarcoplasmic reticular membranes seems to be extremely uncommon. The points of close approximation are often found near the Z line levels of the adjacent myofibrillar sarcomeres. In contracted fibers, the sarcolemma usually is infolded at these levels with a resultant scalloped contour. Porter and Palade [*7*] have suggested that the sarcoplasmic reticulum may function as an anchor between the surface membrane and the Z line structures of the adjacent myofibrils. Deep invaginations of the sarcolemma with attached basement membrane are occasionally found, and may correspond to the apparent branching of myocardial fibers as seen with the light microscope.

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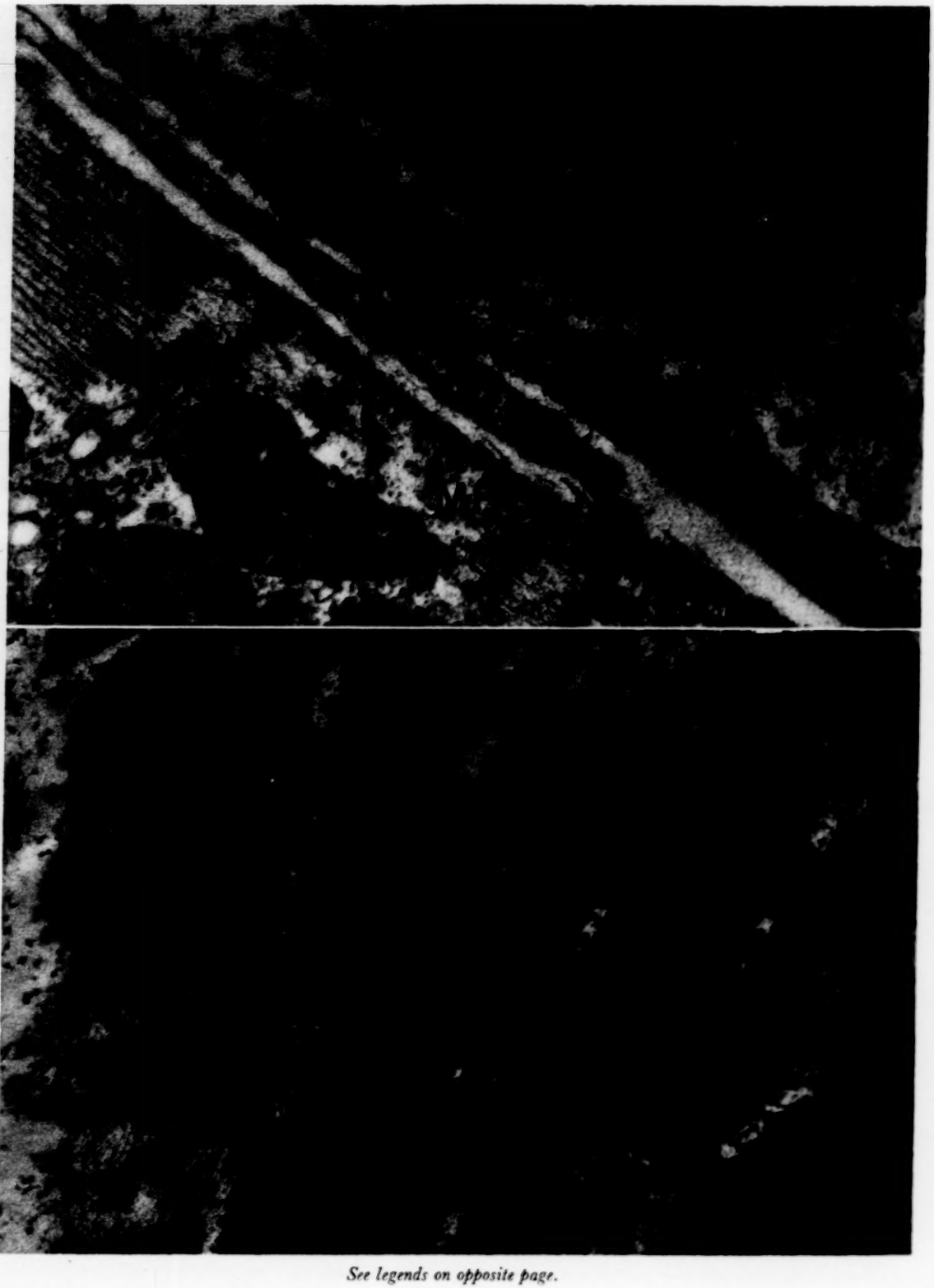

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INTERCALATED DISC

The intercalated disc is defined as a structurally distinctive complex consisting of two apposed limiting cell membranes and an interposed intercellular space. In the present and previous electron microscopic studies, there appears to be no continuity of cell structures across the disc. The concept of a syncytium, as a morphologic entity, would seem to be untenable.

The intercalated disc usually pursues an undulating course, oriented transversely in relation to the long axis of the myofibrils. (Figs. 4 and 5.) Interposed along its course are occasional, irregularly distributed steps of one or several sarcomere lengths in the longitudinal axis, parallel to the myofibrils. (Figs. 5 and 6.)

Although the disc shows three structural variants along its course, the limiting cell membranes are not at any point invested with a basement membrane, and pinocytotic vesicles are not generally evident near the cell surfaces. Segments of the sarcoplasmic reticulum often come into close relationship with the limiting cell membranes of the disc. (Figs. 5 and 6.)

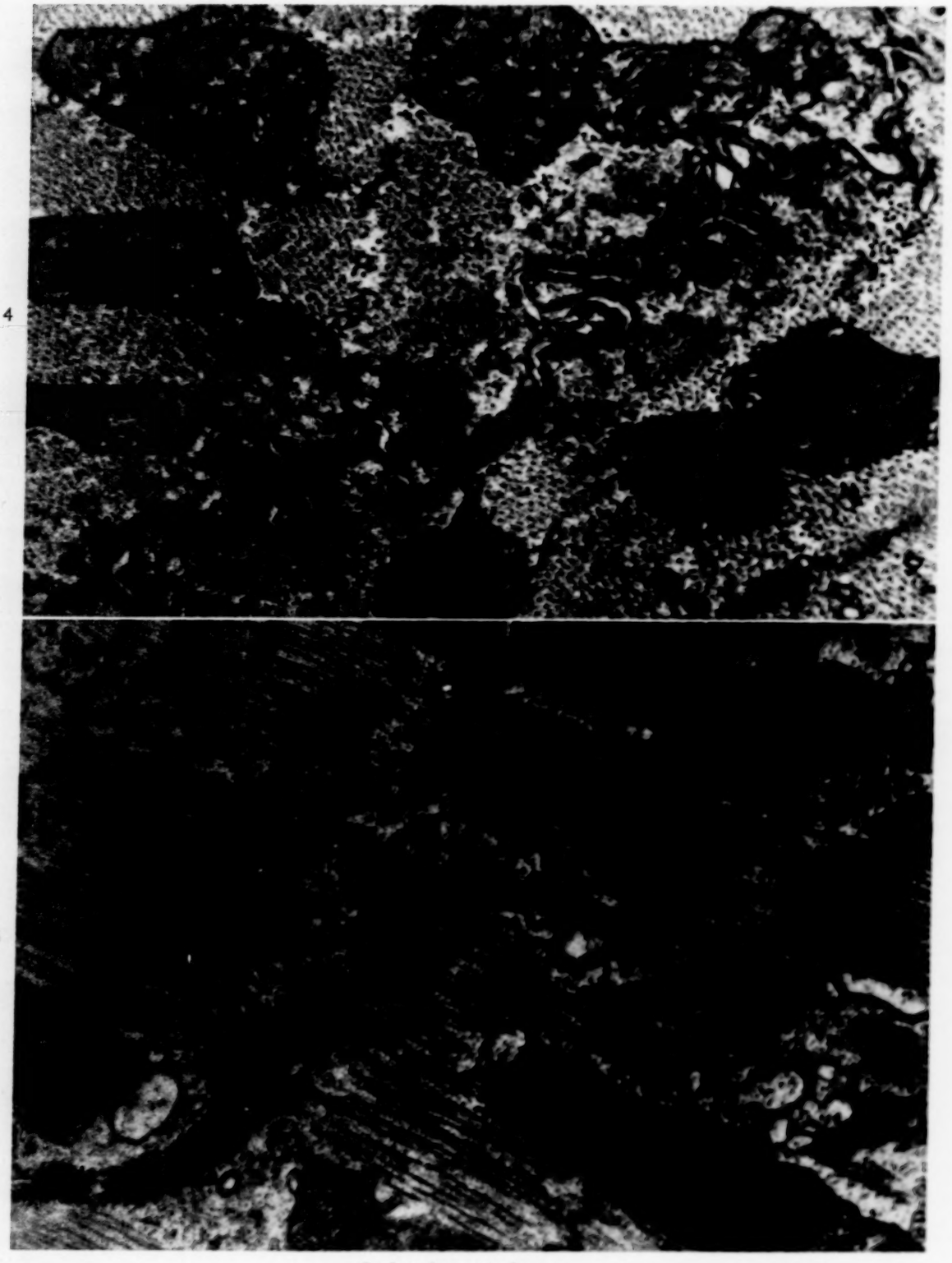
In the first type of disc structure the apposed myocardial cells are limited by thin dense membranes, similar to the sarcolemma; the interposed intercellular space is somewhat variable in width. The myofibrils are usually interrupted by the disc at the Z line level of a sarcomere, and they terminate in a moderately dense deposit extending from the limiting cell membrane of the disc into the cytoplasm. (Fig. 5.) Between myofibrillar terminations, the intracytoplasmic deposit is less evident, or may be entirely absent. This first type is usually transversely oriented, but it may be found in parallel with the long axis of the myofibrils. (Fig. 6.)

The second type of disc modification consists of limiting cell membranes which are more dense than those of the first type. (Figs. 5 and 6.) Here the intercellular space is very narrow and uniform in width. (Fig. 6.) Myofibrils do not terminate in the second type, and there is no contiguous intracytoplasmic deposit. This type is short, compared to the first type, and is usually disposed as a longitudinal link paralleling the long axis of the myofibrils. (Figs. 5 and 6.) The second type is not infrequently found near the origin of an intercalated disc, where a longitudinal orientation may not be evident.

FIG. 3. General cytology. The intercellular space between two contiguous myocardial fibers is narrow in this micrograph. The sarcolemma of each cell is externally covered by a basement membrane. The surface membranes converge on the right at an intercalated disc origin with the second type of disc structure. Subsarcolemmal pinocytotic vesicles, mitochondria, and cytoplasmic granules are demonstrated. At the top of the micrograph is an intermediary vesicle, surrounded by terminal segments of the sarcoplasmic reticulum and containing some opaque material. Original magnification × 50,500.

FIG. 1. General cytology. Intervening between a myocardial fiber and a capillary is a fibrocyte and a scanty amount of collagen. The sarcolemma appears as a thin dense line with an external investing basement membrane. Segments of the sarcoplasmic reticulum are closely related to the sarcolemma. A few pinocytotic vesicles are seen within the myocardial cytoplasm near the sarcolemma. Many pinocytotic vesicles are present in the capillary endothelium. A small portion of an endothelial nucleus is shown in the right upper portion of the micrograph. Capillary = CAP; Myocardial fiber = MF. Original magnification × 54,000.

FIG. 2. General cytology. The sarcolemma appears at the right. The basement membrane adheres to the surface membrane at a shallow invagination. The general structure of a myofibrillar sarcomere is shown. Dense cytoplasmic granules are evident. Z line = Z; I band = I; A band = A; H disc = H. An arrow indicates the M line. Original magnification × 69,000.



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The third type of structural differentiation is represented by desmosomes, scattered irregularly along the course of the first type of disc structure. (Fig. 4.) The desmosomes appear as paired, markedly dense, arcuate bodies lying in apposition on the cytoplasmic aspects of the limiting cell membranes.

Fawcett and Selby [*4*] have previously described desmosomes in relation to the intercalated discs of the turtle atrium. In their material, the desmosomes were located along the lateral surfaces of cardiac muscle cells at Z line levels and were proposed as precursors of the disc structures.

Karrer [*8*] described the intercalated discs in the cardiac-type striated musculature of thoracic and lung veins in mice. Sjöstrand et al. [*9*] have examined the intercalated discs of cardiac muscle in frogs, mice, and guinea pigs. The observations of Karrer and Sjöstrand et al. are fundamentally similar to those herein described although certain structural and interpretive differences deserve comment. Sjöstrand et al. [*9*] describe "longitudinal connecting surfaces," which correspond to our second type of disc structure. Karrer [*8*] uses the term "lateral interconnection," which includes both the second and third types of disc structure delineated herein. These authors limit the term "intercalated disc" to our first type of disc modification.

Karrer [*8*] found small vesicles, presumably pinocytotic vesicles, adjacent to the limiting membranes of the "lateral interconnections." As already noted, our material did not demonstrate pinocytotic vesicles in this location and the desmosomes did not necessarily appear contiguous to the second type of disc structure.

Sjöstrand and his associates [*9*] described five-layered "longitudinal connecting surfaces" in the mouse heart and Karrer [*8*] illustrated "quintuple-layered lateral cell interconnections." Such structures suggest a closer apposition of the limiting cell membranes in these locations than would have been construed from our observations. Because of the intimate relationship of the cell surfaces at these points, Karrer [*8*] postulated a function in cell to cell conduction of electrical impulses. We [*10*] have suggested a similar function. Sjöstrand et al. [*9*] have described "S-regions" along the course of the disc in mouse and guinea pig heart and have proposed that these complex, multilayered structures are concerned with electrical impulse conduction from one cell to the next. "S-regions" may be present in rat and dog cardiac muscle, but they were not well delineated in our study.

MYOCARDIAL NUCLEI

In myocardial fibers, the nuclei are generally centrally located, but occasionally they are near the sarcolemma. The moderately dense, granular, rather uniformly distributed nuclear material is circumscribed by a double perinuclear membrane (Fig. 7), showing an occasional pore complex of the type described by Watson [*11,12*]. Segments of the sarcoplasmic reticulum frequently come into close relationship with the perinuclear membranes. (Fig. 7.) Moore and Ruska [*13*] have observed continuity between the sarcoplasmic reticulum and the outer perinuclear membrane; such direct continuity, however, is difficult to demonstrate. In contracted fibers, the double perinuclear membrane and peripheral portion of the nucleus have a convoluted appearance which is similar to that noted by van Breemen [*14*] in human skeletal muscle.

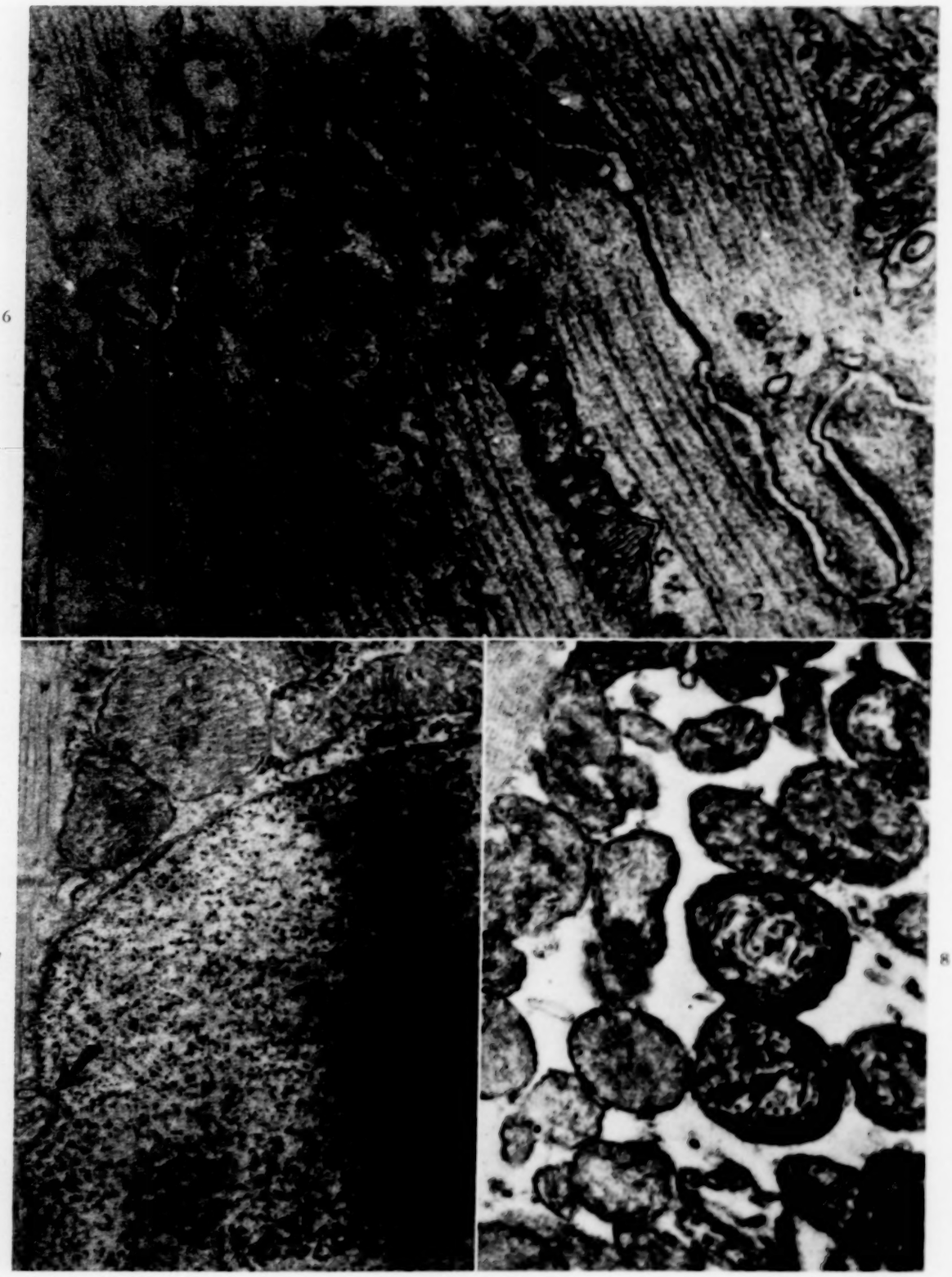
MYOCARDIAL MITOCHONDRIA

Numerous mitochondria are clustered about the nucleus (Fig. 7) or beneath the sarcolemma (Fig. 3) and are widely distributed throughout the myofiber in close proximity to the myofibrils and segments of the sarcoplasmic reticulum. (Figs. 9 and 10.) Rarely an arrangement of one mitochondrion per sarcomere is found along the myofibrils as mentioned by Hodge et al. [*15*]; usually the distribution is less regular.

The mitochondria appear to vary in size but

FIG. 4. Intercalated disc. A transverse section reveals the undulating course of the disc, showing the first type of structural modification over most of its course. Irregularly scattered along the first type are desmosomes (arrows indicate several), the third type of structural differentiation found at the disc. Original magnification × 53,500.

FIG. 5. Intercalated disc. A longitudinal section illustrates the first type of disc structure. The limiting membranes appear as thin dense lines with an intervening intercellular space. The myofibrils terminate at the Z line level in a dense material deposited on the cytoplasmic aspects of the limiting cell membranes. In the mid-portion of the right side of the micrograph is a short segment of the second type of disc structure, which is oriented parallel to the myofibrils. Original magnification × 52,000.



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are generally large. They are delimited by a double membrane and are usually traversed by numerous cristae. Continuity between the inner bounding membrane and the cristae is often observed. The cristae ordinarily do not extend across the entire width of the mitochondria and rarely assume a peculiar spiral or concentric pattern (Fig. 8) as previously described by Moore and Ruska [*13*]. The large number, generally large size, and numerous cristae of myocardial mitochondria are not surprising in such metabolically active tissue.

SARCOPLASMIC RETICULUM

Following the concept developed and graphically depicted by Porter and Palade [*7*], we interpret the sarcoplasmic reticulum as a system of anastomosing, membrane-limited channels which traverse the interior of the myocardial cells. This system is highly developed in the myocardium and is predominantly smooth-surfaced or agranular in type. A few rough-surfaced or granular segments are found near the nucleus.

In electron micrographs, the sarcoplasmic reticulum usually appears as a series of closely related vesicles and tubules, although favorable sections reveal the underlying anastomosing pattern. (Fig. 9.) The limiting membranes of the reticulum appear as thin, dense lines, and the delimited areas may be of the same density as the surrounding cytoplasm or may be lighter or darker than the cytoplasm.

Close apposition of segments of the sarcoplasmic reticulum to other cell structures is often observed. Relationships of the reticulum to the sarcolemma, intercalated disc, perinuclear membranes, and mitochondria have been noted herein. The myofibrils are closely embraced by the sarcoplasmic reticulum, which often appears to have transverse continuity from one myofibril to the next, at or near the Z line level. (Fig. 9.) A transverse component at the level of the H disc is sometimes observed. (Fig. 9.) Opposite the A and I band levels, the sarcoplasmic reticulum tends to parallel the long axis of the myofibrils.

Longitudinal continuity of the sarcoplasmic reticulum generally appears to be limited to the length of a single sarcomere, with only rare extensions of channels across Z line levels. Usually segments of the sarcoplasmic reticulum from adjacent sarcomeres appear to terminate in close apposition near the Z line level. An intermediary vesicle may be interposed between these terminal segments (Figs. 3, 9 and 10) with the formation of a "triad" as defined by Porter and Palade [*7*].

In rat and dog heart muscle, the intermediary vesicle appears as a spherical, ovoid, or slightly irregular structure, bounded by a single dense membrane. (Fig. 10.) There seems to be no continuity between the intermediary vesicle and the adjacent segments of the sarcoplasmic reticulum, although the terminal reticular segments are often closely applied to the surface of the intermediary vesicle. (Figs. 3 and 10.) In myocardium these vesicles are generally large and single in contrast to the small, double intermediary vesicles often encountered in skeletal muscle. While ordinarily appearing devoid of content, the myocardial intermediary vesicles occasionally contain opaque material (Fig. 3) and rarely appear to include one or several tiny subvesicles. (Fig. 9.)

In addition to the anchoring function mentioned, Porter and Palade [*7*] proposed two other functions for the sarcoplasmic reticulum: (1) the channels may serve as a conduit for the transport of metabolites to and from the interior of the cell, and (2) the limiting membranes may be important in the conduction of an electrical impulse into the interior of the cell. Both proposals seem reasonable and the latter receives support from the work of Huxley and Taylor [*16*]. The sarcoplasmic reticulum has also been suggested as the intracellular site of relaxing factor synthesis or storage [*22*].

FIG. 6. Intercalated disc. A segment of a disc shows the first and second types of disc structure. The second type reveals limiting membranes which appear more dense than those of the first type. The intervening intercellular space at the second type is narrow and uniform in width. Segments of the sarcoplasmic reticulum come into close relationship with the first type of disc modification. Original magnification × 76,500.

FIG. 7. Myocardial nucleus. A segment of a nucleus demonstrates the double perinuclear membrane. Closely related segments of the sarcoplasmic reticulum are seen near the arrow. Mitochondria are evident in the upper portion of the picture. Original magnification × 28,000.

FIG. 8. Myocardial mitochondria. In the cluster of mitochondria illustrated, two demonstrate a spiral or concentric arrangement of the cristae. Original magnification × 27,000.

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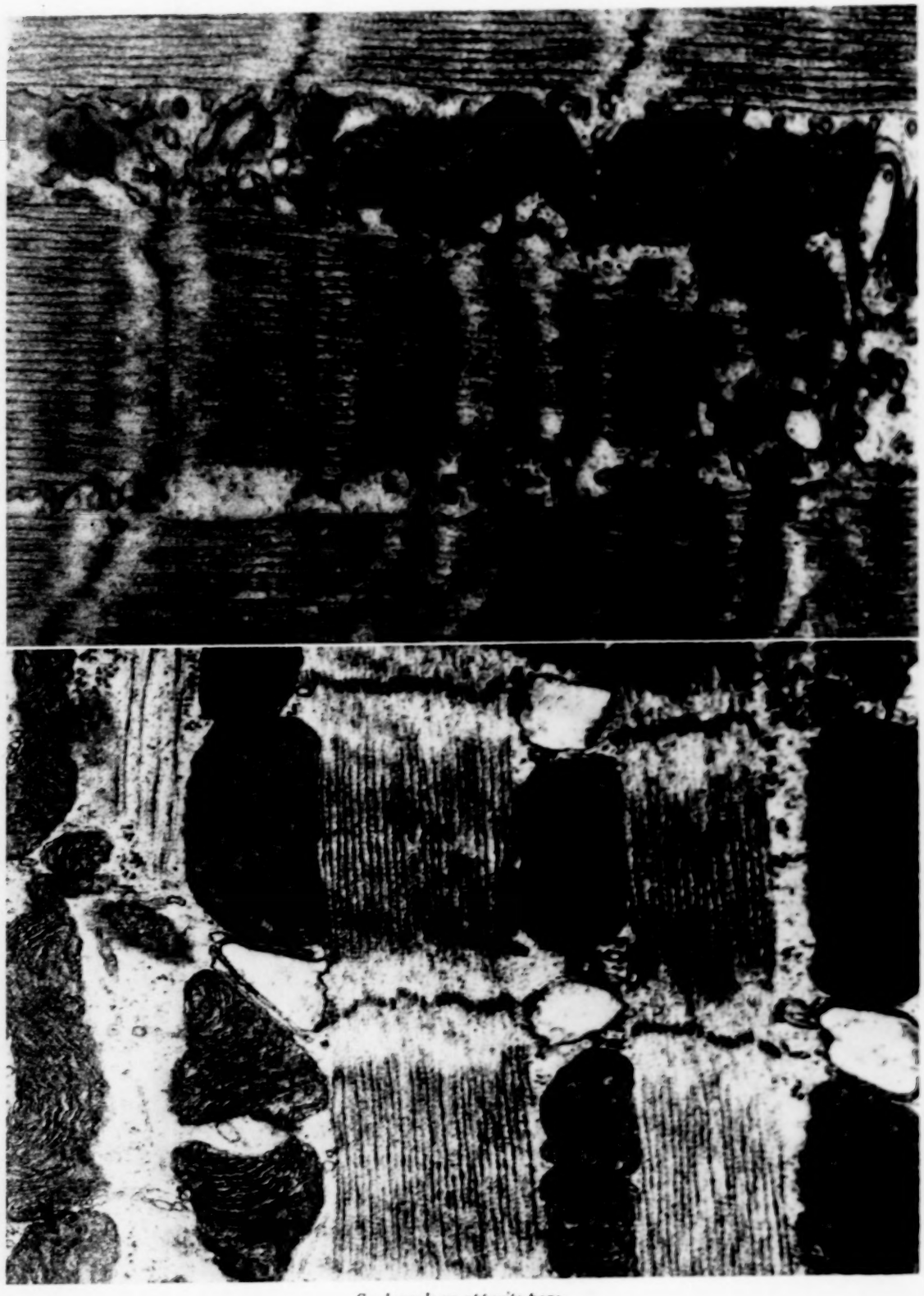

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MYOFIBRILS

The cardiac myofibril is conceived as an ordered, integrated system of overlapping thick and thin myofilaments. Huxley [17] has described a similar myofibrillar structure in skeletal muscle. In a mature myocardial cell, there are numerous myofibrils oriented parallel to the long axis of the cell and occasionally appearing to branch. Mitochondria and segments of the sarcoplasmic reticulum are frequently observed between the myofibrils.

The structural unit of the myofibril is the sarcomere, defined as that portion lying between two consecutive Z lines. The sarcomere has a banded appearance due to the interrelationships of the thick and thin myofilaments. In optimal, rest length or slightly stretched, heart muscle, the sarcomere consists of two limiting Z lines, adjacent lateral I bands, intermediate A bands, and a central H disc bisected by an M line. (Figs. 2 and 11.)

In ultrathin longitudinal and transverse sections, the Z lines and I bands appear to contain only thin myofilments. (Figs. 2, 11, 13 and 14.) The ordered array and the longitudinal orientation of the filaments is better preserved at the Z line than in the I band. (Fig. 13.) The Z line is further characterized by an unresolved background density. (Figs. 2 and 13.) The A bands consist of interdigitated thick and thin filaments. (Figs. 11, 12, 14 and 16.) Crossbridges are observed as thin transverse links between the myofilaments. (Fig. 16.) The H disc and M line demonstrate only thick filaments in an orderly hexagonal array. (Fig. 15.) Longitudinal sections show that the M line is constituted by nodular thickenings of the thick myofilaments. (Fig. 2.) Transverse sections occasionally reveal thin transverse crossbridges linking the thick filaments together at the M line. (Fig. 15.)

Transverse sections of the A band reveal a highly ordered structure with the thick filaments disposed in a primary hexagonal array, and the thin filaments regularly arranged at trigonal points in relation to the thick filaments.

11 12

FIG. 11. Myofibril (longitudinal section). Only thin filaments appear in the I bands; only thick filaments at the H disc. In the A bands the thick and thin filaments interdigitate. One thin filament is interposed between two thick filaments in this sarcomere. Z line = Z; I band = I; A band = A; H disc = H. The nodular thickenings at the center of the sarcomere identify the M line. Original magnification × 74,000.

FIG. 12. Myofibril (longitudinal section). Similar to Figure 11 except that two thin filaments are interposed between contiguous thick filaments in the A band. This is most evident at the arrows. Original magnification × 72,500.

(Fig. 16.) Thus, in transverse sections, six thin filaments are arrayed about a single thick filament. (Figs. 14 and 16.) In ultrathin longitudinal sections of the A band, under optimal conditions, either 1:1 or 1:2 alterations of thick to thin filaments are observed. In the former, one thin filament is interposed between two

FIG. 9. Sarcoplasmic reticulum. In the upper left portion of the micrograph, the reticulum appears as a closely related series of tubules and vesicles. In the lower right portion, the anastomosing pattern of the sarcoplasmic reticulum is evident. Transverse components at the Z line and in the region of the H disc are noted. In the upper right portion is an intermediary vesicle closely related to terminal segments of the sarcoplasmic reticulum and containing a tiny subvesicle. Original magnification × 51,500.

FIG. 10. Sarcoplasmic reticulum. Four intermediary vesicles are observed. The tendency for these to appear at Z line levels is evident. Original magnification × 50,000.

FIG. 13. Myofibrils (transverse section). The different appearance of the I and A bands is apparent, even at this magnification. The thin filaments appear in a more ordered array at the Z line than in the I band. Z line = Z; I band = I; A band = A. Original magnification × 52,500.

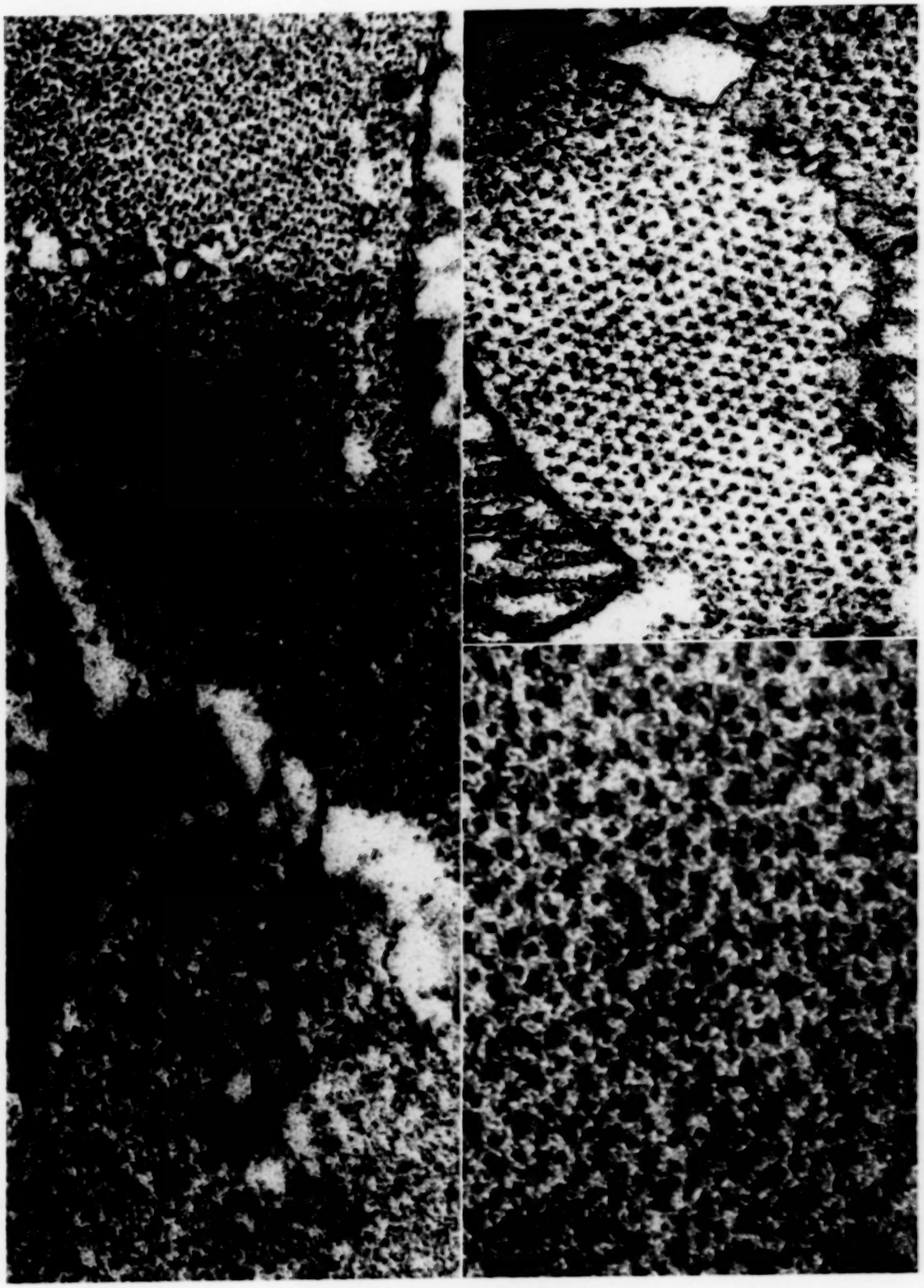
thick filaments (Fig. 11); in the latter two thin filaments are interposed between the thick filaments. (Fig. 12.) The reasons for these appearances in longitudinal sections have been elaborated by Huxley [*17*].

Under favorable conditions continuity of the thin filaments from the Z line through the I band and into the A band may occasionally be observed in longitudinal sections. (Fig. 12.) The thin filaments may appear to extend across the Z line into the I band of the contiguous sarcomere. (Fig. 2.) Similar material often demonstrates continuity of the thick filaments through both A bands, the H disc, and the M line. (Figs. 11 and 12.) Furthermore, in transverse sections, filament counts reveal number ratios of 2:3:1 for I band:A band:H disc, respectively. On the basis of this evidence it is concluded that the structural arrangements of the thick and thin myofilaments in cardiac muscle are entirely similar to those observed by Huxley [*17*] in skeletal muscle. Thus, in rest-length or slightly stretched cardiac muscle, the sarcomere consists of an orderly arrangement of

FIG. 14. Myofibrils (transverse section). The appearance of the I and A bands is shown. Only thin filaments are present in the I band shown at the bottom of the picture. The A band in the upper portion of the micrograph reveals interdigitating thick and thin filaments. The thin filaments are smaller and more dense than the thick filaments. In many areas of the A band an arrangement of six thin filaments about a single thick filament can be identified. Original magnification × 105,000.

FIG. 15. Myofibril (transverse section). At the H disc and M line only thick filaments are observed. Slender cross bridges link the thick filaments together at the M line. Original magnification × 107,000.

FIG. 16. Myofibril (transverse section). In the A band shown, interdigitating thick and thin filaments can be discerned. The thick filaments are disposed in a primary hexagonal array. The thin filaments appear at trigonal points in relation to the thick filaments. Thus, in many areas, six thin filaments can be counted around a single thick filament. Several encircled areas are included to emphasize this relationship, which can be easily obscured by slight obliquity of the section or confusion of sectioned crossbridges with myofilaments. Original magnification × 265,000.



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thick and thin myofilaments which interdigitate in the A band. The thin filaments extend from the Z lines to the nearest A-H junction. The thick filaments traverse the central portion of the sarcomere from one A-I junction to the other.

Huxley and Hanson [*18,19*] and Huxley and Niedergerke [*20*] have proposed an interesting model for muscle contraction. This model, sometimes called the sliding filament hypothesis, involves the movement of thin filaments relative to the thick filaments during muscle contraction. The thin filaments were proposed as actin; the thick filaments as myosin. During the contractile process the thin filaments were considered to move further toward the center of the sarcomere, with a consequent shortening of the I bands and H disc and therefore of the sarcomere as a whole. The morphologic and other evidence favoring this model have been summarized by Huxley and Hanson [*21*] and Huxley [*22*]. The major objections to the sliding filament hypothesis have been cited by Hodge [*23*]. Further investigation seems to be indicated before this model of muscle contraction can be accepted. Meanwhile, it can be stated that, whatever its functional significance may be, the myofibrillar structure of cardiac muscle is entirely comparable to that of skeletal muscle. Karrer [*24*] has shown a similar structure in striated muscle of blood vessels. Recently cholinesterase activity has been demonstrated in the M lines of diaphragmatic and cardiac muscle [*25*].

MYOCARDIAL CYTOPLASM

The cytoplasm of the myocardial fibers consists of a slightly dense background within which are occasional large dense or opaque bodies and numerous small dense granules. (Figs. 2 and 3.) The large bodies are generally construed to represent lipid. The small dense granules were originally proposed as ribonucleoprotein particles by Palade [*26*], who suggested that these loose granules were similar in nature to the granules attached to the rough-surfaced type of endoplasmic reticulum. More recently Fawcett and Selby [*4*] have proposed that the unattached cytoplasmic granules of heart muscle represent a particulate form of glycogen. We favor the latter viewpoint and note that the unattached granules in rat myocardium appear to be smaller than those of dog myocardium and, in this respect, differ from the ribonucleoprotein particles described in other locations. The precise nature of these unattached cytoplasmic granules awaits further study.

SUMMARY

1. An electron microscopic study of the papillary muscles of rat and dog hearts is reported. The ultrastructural features of the sarcolemma, intercalated disc, myocardial nuclei and mitochondria, sarcoplasmic reticulum, myofibrils, and other cytoplasmic components are described.

2. The observations of other investigators and their interpretations are discussed in relation to these findings.

3. The intercalated disc is defined as a distinctive complex consisting of modified cell membranes with an interposed intercellular space. The concept of a morphologic syncytium is discarded.

4. The sarcoplasmic reticulum is interpreted as a system of anastomosing, membrane-limited channels which traverse the interior of the myocardial cell and come into close relationship with other intracellular structures.

5. The myofibril is conceived as an ordered integrated system composed of thick and thin myofilaments which interdigitate at A band levels. The structural similarity to skeletal muscle is discussed.

Acknowledgment: We thank Dr. Benjamin Castleman for his constant encouragement and support. The excellent technical assistance of Miss Elizabeth Johnson is gratefully acknowledged. Dr. Henry deF. Webster and Dr. James B. Caulfield gave very helpful advice on many aspects of this study.

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Pathways of Metabolism in Heart Muscle*

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THE metabolic activities in cardiac muscle reflect the function which the heart performs. Because the physiological role of the heart is fulfilled in the performance of mechanical work, the metabolic events which are of utmost importance are those concerned with the production of energy in a form that is readily available for use in mechanical activity. Because of the extraordinary amount of energy required by the heart, the organization and function of myocardial metabolism are geared to the production of energy on a large scale. The *synthetic* processes which occur in heart muscle are largely restricted to those required for sustaining the integrity of the heart itself. An understanding of cardiac metabolism can therefore follow only from a clear conception of energy production in the myocardial cell.

The energy utilized for the work output of the contracting myocardium is produced by the trapping of some of the energy released during the enzymic degradation of foodstuffs to carbon dioxide and water. By the process of oxidative phosphorylation this energy is captured in the high energy phosphate bond of ATP, a form in which it can be readily utilized. The complex system of enzymes and cofactors necessary for the process of oxidative phosphorylation has been found to operate exclusively within one subcellular organelle, the mitochondrion. This observation reflects, on a biochemical level, the cytological finding that the myocardial cell contains an unusually large number of mitochondria. The fact that the entire machinery for energy production functions within the mitochondrion underlies the key position of this subcellular structure in myocardial metabolism.

Before a detailed discussion of mitochondrial energy production is undertaken, it will be helpful to consider briefly the intermediary metabolism of the various foodstuffs as it applies to myocardial function. In this way the mechanisms by which the substrates for energy production are derived from carbohydrate, fat and amino acids will be formulated.

METABOLISM OF CARBOHYDRATE AND THE CITRIC ACID CYCLE

The degradation of carbohydrate to carbon dioxide and water is accomplished in two stages: anaerobic and aerobic. In the first stage, which takes place in the cytoplasm of the cell, glucose or glycogen is degraded anaerobically to pyruvate by the series of enzymic reactions outlined in Figure 1.† In the course of these reactions four molecules of ATP are produced per molecule of glucose degraded. (Fig. 1, reactions ③ and ④.) These phosphorylations are carried out by an interaction between substrate and phosphate acceptor—a process by which a high energy phosphate is transferred from the substrate directly to ADP. Since two molecules of ATP are used up during glycolysis (Fig. 1, reactions ① and ②), the net yield of ATP is only two molecules per molecule of glucose consumed. The pyruvate which is formed may be hydrogenated to form lactate, a reaction catalyzed by lactic dehydrogenase in which the hydrogen donor is reduced diphosphopyridine nucleotide (DPNH). Pyruvate has, however, an alternative fate: entrance into the mitochondrial system of aerobic metabolism in which it may be degraded completely to carbon dioxide and water. Part of the pyruvate utilized by the myocardial cell is not produced within that cell, but is produced elsewhere in the body and brought to the myocardium by the circulating blood. Likewise, lactate is largely supplied preformed to the myocardium.

The aerobic phase of carbohydrate catabolism consists of that series of enzymic reactions known as the citric acid cycle [2] and takes place entirely within the mitochondrion. (Fig. 2.) Actually, the citric acid cycle should also be considered the second, or aerobic, phase of fatty acid and amino acid catabolism. It is discussed at this point only for the sake of convenience.

† Glycolysis is not the only pathway for the degradation of carbohydrates. A comprehensive review of this subject has been recently published [1].

* From the Institute for Enzyme Research, University of Wisconsin, Madison, Wisconsin.

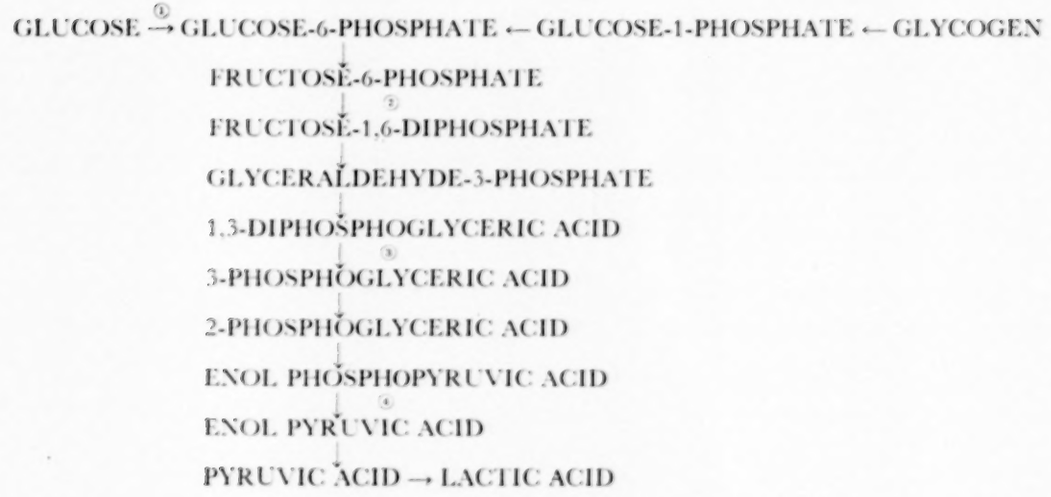


FIG. 1. Glycolysis: the anaerobic phase of carbohydrate catabolism.

In the citric acid cycle, five of the chemical reactions are accompanied by the production of ATP from ADP and inorganic phosphate. They are all oxidations, and hence the term *oxidative phosphorylation*. The five oxidations are linked to phosphorylation through the reductions accompanying them. The electrons which are released during these oxidations are passed along through a series of enzymes and coenzymes which are collectively known as the mitochondrial electron transport system. It is in the shuttling of electrons through this system that the esterification of inorganic phosphate is accomplished. The five citric acid cycle oxidations which may be coupled to phosphorylation are (1) the oxidation of pyruvate to acetyl-CoA and carbon dioxide; (2) the oxidation of isocitrate to α-ketoglutarate and carbon dioxide; (3) the oxidation of α-ketoglutarate to succinyl-CoA and carbon dioxide; (4) the oxidation of succinate to fumarate; and (5) the oxidation of malate to oxaloacetate. (Fig. 2, reactions ① to ⑤.) Four of the five reactions require the participation of a pyridine nucleotide to pass on to the electron transport system the electrons which are released. The exception is the oxidation of succinate to fumarate, in which the electrons released from succinate may enter the electron transport system through a direct route which will be discussed later.

The degradation of one molecule of pyruvate through the citric acid cycle yields fifteen molecules of ATP. For each molecule of pyridine nucleotide reduced, three molecules of ATP are formed by the entrance of electrons from the reduced pyridine nucleotide into the mitochondrial electron transport system. Since the reduction of pyridine nucleotide occurs in four of the five oxidations mentioned, the yield of ATP from these four oxidations amounts to twelve molecules. In addition, two molecules of ATP are produced by the oxidation of succinate, and one other molecule of ATP is formed by a direct (substrate-linked) phosphorylation as α-ketoglutarate is converted to succinyl-CoA. Thus the total yield of ATP comes to fifteen molecules per molecule of pyruvate, or thirty per molecule of glucose [*3,4*]. Comparison of the latter figure with the two molecules of ATP produced during the anaerobic degradation of glucose vividly contrasts the relative importance of the two phases of carbohydrate catabolism in the production of energy for cellular work.

FAT METABOLISM

The first step in the enzymic degradation of fat is a hydrolytic process yielding glycerol and fatty acids. This step is not required for the utilization of the free fatty acids conveyed to the heart by the circulating blood. For the utilization of fat stored within the myocardium, however, and for the metabolism of the fat which is brought to the heart as such, hydrolysis is an obligatory first step. Likewise, a reversal of the process of fat cleavage is necessary for the conversion of fatty acids to fat for storage within the myocardium. The glycerol formed by the hydrolysis of fat may be oxidized to glyceraldehyde which, in turn, may continue on the path of intermediary metabolism for triose sugars out-

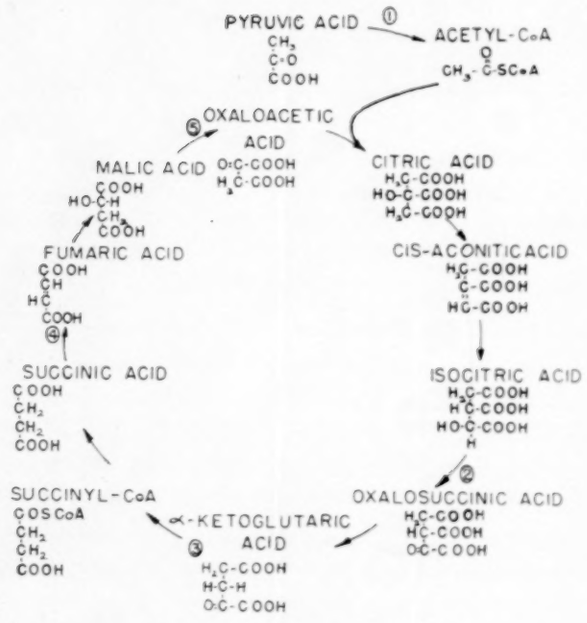


FIG. 2. The citric acid cycle.

lined in Figures 1 and 2. The fate of the fatty acid residues which are formed by the hydrolytic cleavage of fat is far more complex.

All the enzymes required for degradation of fatty acids to carbon dioxide and water are contained in the mitochondrion [*5,6*]. Direct proof for the mechanism of fatty acid oxidation was obtained by reconstruction of the process through isolation of the system of soluble enzymes from mitochondria [*7–9*]. The process has turned out to be a cyclic one substantially the same as originally proposed by Knoop over fifty years ago [*10*]. There are five steps in the cycle, the over-all effect of which is the detachment of a two-carbon unit from the original fatty acid, with the formation of a new fatty acid which is two carbon atoms shorter in length:

$$RCH_2CH_2COOH + 2H_2O \xrightarrow{-4H} RCOOH + CH_3COOH \quad (1)$$

The cycle is repeated again and again until the original fatty acid has been whittled away to a fragment which itself contains only two or three carbon atoms. The two-carbon fragment formed in each turn of the cycle is degraded completely to carbon dioxide and water by entering the citric acid cycle as acetyl-CoA. Details of the five steps are summarized in Table 1. The first step is the formation of the coenzyme A ester of the free fatty acid in the presence of ATP to form an acyl-CoA (equation 1). Next, the acyl-CoA is oxidized by a flavoprotein (acyl dehydrogenase) to form the corresponding α,β-unsaturated derivative (equation 2). In this reaction the electrons removed by the acyl dehydrogenase are passed on to another flavoprotein enzyme known as the electron transferring flavoprotein (ETF) and from ETF into the mitochondrial electron transport system, where the flow of electrons is coupled to the formation of ATP [*11*]. The next step in the fatty acid oxidation cycle is hydration of the unsaturated compound to form the corresponding β-hydroxyacyl-CoA derivative (equation 3). Another dehydrogenation follows, and the electrons released are this time passed on to a pyridine

TABLE 1

FATTY ACID OXIDATION CYCLE*

Equation 1 $R{-}CH_2{-}CH_2{-}\overset{\overset{O}{\|}}{C}{-}OH + CoASH + ATP \rightarrow R{-}CH_2{-}CH_2{-}\overset{\overset{O}{\|}}{C}{-}SCoA + AMP + 2Pi$

Equation 2 $R{-}CH_2{-}CH_2{-}\overset{\overset{O}{\|}}{C}{-}SCoA \xrightarrow[-2H]{\text{acyl dehydrogenase}} R{-}CH{=}CH{-}\overset{\overset{O}{\|}}{C}{-}SCoA$

Equation 3 $R{-}CH{=}CH{-}\overset{\overset{O}{\|}}{C}{-}SCoA + H_2O \rightarrow R{-}\underset{\underset{OH}{|}}{CH}{-}CH_2{-}\overset{\overset{O}{\|}}{C}{-}SCoA$

Equation 4 $R{-}\underset{\underset{OH}{|}}{CH}{-}CH_2{-}\overset{\overset{O}{\|}}{C}{-}SCoA + DPN \rightarrow R{-}\overset{\overset{O}{\|}}{C}{-}CH_2{-}\overset{\overset{O}{\|}}{C}{-}SCoA + DPNH + H^+$

Equation 5 $R{-}\overset{\overset{O}{\|}}{C}{-}CH_2{-}\overset{\overset{O}{\|}}{C}{-}SCoA + CoASH \rightarrow R{-}\overset{\overset{O}{\|}}{C}{-}SCoA + CH_2{-}\overset{\overset{O}{\|}}{C}{-}SCoA$

* The following abbreviations are used in this table: CoASH (coenzyme A); ATP (adenosine-triphosphate); AMP (adenosine-monophosphate); Pi (inorganic phosphate); and DPN and DPNH (oxidized and reduced forms of diphosphopyridine nucleotide).

nucleotide (DPN) (equation 4). The DPNH formed in this reaction is oxidized in the mitochondrion, and the electrons thereby removed from it pass through the electron transport system to yield three molecules of ATP. The final step in the fatty acid oxidation cycle is a cleavage that results in the formation of acetyl-CoA and the coenzyme A derivative of a new fatty acid which is two carbon atoms shorter than the original one (equation 5). The new fatty acid re-enters the cycle in the reaction outlined in equation 2, since it is already esterified with coenzyme A. Thus the molecule of ATP required for the formation of the coenzyme A ester of the original fatty acid is the only one used in the entire process of fatty acid degradation.

Acetyl-CoA, the two-carbon unit which is formed with each turn of the fatty acid oxidation cycle, condenses with oxaloacetate, in the presence of a specific enzyme [*12*], to form citrate, which undergoes oxidation in the citric acid cycle. If the original fatty acid contained an even number of carbon atoms, then the last fragment will itself be acetyl-CoA. If the original fatty acid contained an odd number of carbon atoms, then the last fragment will be propionic acid, which may be converted directly to succinic acid (a citric acid cycle substrate) by carbon dioxide fixation [*13*]. The degradation of fatty acids is thus linked to the production of ATP by four routes: (1) through the electron transferring flavoprotein, by which electrons removed in the second step of the cycle are fed into the electron transport system; (2) through DPN, by which electrons removed in the fourth step of the cycle are fed into the electron transport system; (3) through the production of acetyl-CoA, which feeds into the citric acid cycle by condensation with oxaloacetate; and (4) through the production of propionic acid which, by carbon dioxide fixation, can enter the citric acid cycle as succinate. The functional links between the fatty acid oxidation cycle and the citric acid cycle point up the importance of the proximity of these two metabolic systems, both of which are wholly contained within the mitochondrion [*6,9,14*].

The process of fatty acid oxidation cannot proceed unless the citric acid cycle is functioning concomitantly. The reason for this is twofold. First, unless ATP is generated by the citric acid cycle, the first step of the fatty acid oxidation process (Table 1, equation 1) cannot occur. Secondly, when no oxaloacetate is produced, no condensing partner is available for the acetyl-CoA formed by fatty acid oxidation. The ensuing accumulation of acetyl-CoA would make free coenzyme A unavailable, and hence prevent further fatty acid oxidation. The condensation of two molecules of acetyl-CoA to form acetoacetyl-CoA, and the deacylation of this compound to form free acetoacetate plus free coenzyme A is not possible in heart muscle; that process is specific to the liver [*9*]. Free acetoacetate may, however, be conveyed to the heart from the liver and be metabolized by the myocardial cell by formation of its coenzyme A derivative and cleavage to two molecules of acetyl-CoA. It may also arise from the oxidation of β-hydroxybutyrate, which is an energy yielding process in which DPNH is produced concomitantly:

$$CH_3-\underset{\displaystyle OH}{\underset{|}{CH}}-CH_2-COOH + DPN \rightarrow CH_3-\overset{\displaystyle O}{\overset{\|}{C}}-CH_2-COOH + DPNH + H^+ \qquad (2)$$

AMINO ACID METABOLISM

The contribution of amino acid metabolism to energy production in the myocardium is by no means unimportant [*15*]. The mechanism of this contribution is basically the same as that already discussed for carbohydrate and fat, since amino acids yield ATP by undergoing degradation in the citric acid cycle. The problem, then, is the mode of transformation of amino acids into citric acid cycle substrates.

The formation of an α-keto acid from the corresponding amino acid may be accomplished by transfer of the α-amino group to another α-keto acid [*16*]. This process of transamination is catalyzed by enzymes which are specific for the particular pair of acids involved in the reaction [*17*], and requires the participation of pyridoxal phosphate as a cofactor [*18,19*]:

$$R-\underset{\displaystyle NH_2}{\underset{|}{CH}}-COOH + R'-\overset{\displaystyle O}{\overset{\|}{C}}-COOH \xrightarrow{\text{transaminase}} R-\overset{\displaystyle O}{\overset{\|}{C}}-COOH + R'-\underset{\displaystyle NH_2}{\underset{|}{CH}}-COOH \qquad (3)$$

TABLE II
DIRECT TRANSFORMATION OF AMINO ACIDS TO CITRIC ACID CYCLE SUBSTRATES

Amino Acid	Enzymic Process	α-Keto Acid Formed (citric acid cycle substrate)
Alanine	Transamination	Pyruvic acid
Glutamic acid	Transamination	α-Ketoglutaric acid
Aspartic acid	Deamination	Fumaric acid
Aspartic acid	Transamination	Oxaloacetic acid
Serine	Dehydration and deamination	Pyruvic acid

TABLE III
GLYCOGENIC AND KETOGENIC AMINO ACIDS

Glycogenic Amino Acids*	Ketogenic Amino Acids
Threonine	Leucine
Valine	Isoleucine
Cystine	Phenylalanine
Glycine	Tyrosine
Histidine	
Arginine	
Lysine	
Methionine	
Isoleucine	

* In addition to those listed in Table II.

Other but less important pathways exist for the conversion of an amino acid to the corresponding α-keto acid [*20*]. By thus losing the α-amino group certain amino acids can enter directly into the citric acid cycle. Examples of this phenomenon are listed in Table II.

Other amino acids, after losing their amino group, enter into the citric acid cycle only after further chemical transformation, a detailed discussion of which is beyond the scope of this review. In general, they may be divided into two groups, depending upon whether they eventually give rise to acetoacetic acid and other ketone bodies (which are metabolized as described in the preceding section), or to pyruvate or propionic acid (which may be converted to succinate by carbon dioxide fixation, and thence metabolized via the citric acid cycle). The former are called ketogenic; the latter, together with those amino acids listed in Table II, are called glycogenic. Some of the amino acids in each group are listed in Table III.

It should be apparent from the foregoing review of intermediary metabolism that degradation of the various foodstuffs to carbon dioxide and water proceeds in two stages. The first stage is the production of certain key molecules of small size which are common to all three of the foodstuffs. This stage consists of glycolysis in the case of carbohydrates; hydrolysis and fatty acid oxidation in the case of fats; and deamination and other biochemical transformations in the case of amino acids. The second stage consists of the citric acid cycle, where the small molecules produced in the first stage are degraded completely to carbon dioxide and water. It is in this second stage that the major part of energy production is accomplished.

THE MITOCHONDRION

Function. The coupling of the oxidation of citric acid cycle substrates to the production of ATP occurs only within the mitochondrion.* When one considers the enormous complexity of this process, it is not surprising that it should proceed in a highly organized, compact structure. Also localized within the mitochondrion is the entire system for the oxidation of fatty acids, which is functionally linked to the citric acid cycle. In addition to the degradative pathways related to energy production, the mitochondrion contains systems for the synthesis of phospholipid [*21*], protein [*22*] and other substances. The synthetic processes are, however, of relatively minor importance in the case of the heart muscle mitochondrion, where energy production seems to take precedence over all other functions.

The mechanism by which mitochondrial oxidations lead to the synthesis of ATP is not a simple one. A chemical oxidation is, by definition, always accompanied by a reduction. The change which occurs when an oxido-reduction takes place is the transference of electrons from the substance undergoing oxidation to the one undergoing reduction. An example of this process is the oxidation of malate which, by giving up electrons, is converted to oxalacetate, while DPN, by accepting electrons, undergoes reduction to form DPNH. DPNH is in turn oxi-

* There are, in addition, other oxidations which are linked to the production of ATP within the mitochondrion but which have no connection with the citric acid cycle. Examples of this phenomenon are the oxidation of β-hydroxybutyric acid (see formula 2), and the second and fourth steps in the fatty acid oxidation cycle (Table I, equations 2 and 4).

dized back to DPN and the electrons thereby removed from it are transferred to another compound, and from this compound to still another. Thus the electrons originally removed from malate are handed, like water in a bucket brigade, through a complex system of enzymes and coenzymes, and finally reach oxygen. During this shuttling of electrons through what is known as the electron transport system, the series of oxido-reductions is accompanied by the release of energy. The energy released during some of the oxido-reductions of the electron transport system is lost as heat. But in a few specific oxido-reductions, this energy is captured by the esterification of inorganic phosphate, and preserved as the bond energy of ATP.

The concept of the mitochondrion as a complete system in which the citric acid cycle and the electron transport system function in conjunction with the production of ATP was slowly accepted by the scientific world, but the supporting evidence for this view has by now grown so great as to be unequivocal [*23*]: isolated intact mitochondria can carry out oxidative phosphorylation in the presence of oxygen when supplemented with only an appropriate substrate, ADP and inorganic phosphate. Thus the mitochondrion is a complete biochemical unit which contains all the parts necessary for the large number of catalytic functions it performs [*5,6,14*].

Structure. Together with the wider concept of the mitochondrion as a complete biochemical unit came the necessity for change in the biochemical view of structural organization. The original concept of the mitochondrion as a bag within which many components float around, reacting together whenever they happen to collide in the right combination, has proved to be naive. Such disorganization is unthinkable when one considers the diversity of individual reactions that are geared together in a highly complex system for the efficient performance of only a few large biochemical functions.

The early ideas of mitochondrial structure were, of necessity, gross outlines of size and shape. With the application of electron microscopy to the study of the mitochondrion, a more detailed picture could be obtained [*24–26*]. By the integration of biochemical procedures for subdividing mitochondria with the use of recent technics in electron microscopy, a meaningful picture of the substructure of the mitochondrion is now emerging.

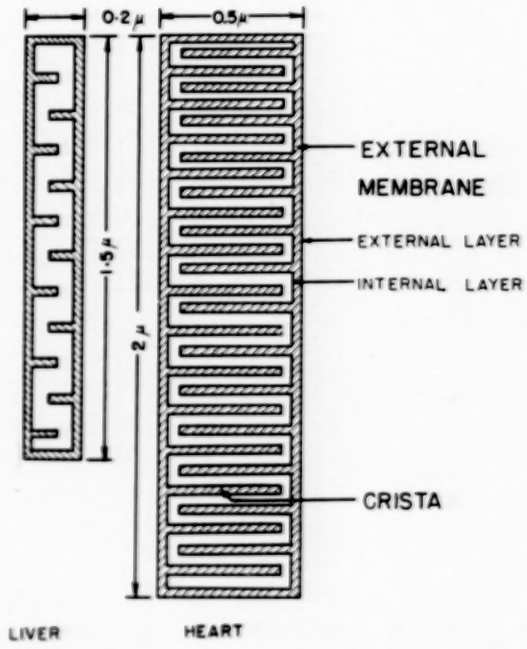


Fig. 3. Idealized drawings of mitochondria of liver and heart muscle, showing relative sizes and relative concentrations of cristae.

The heart muscle mitochondrion is a rod-shaped body, approximately 2 μ in length and 0.5 μ in width. An idealized drawing of a heart muscle mitochondrion and one of a liver mitochondrion are presented in Figure 3. An electron photomicrograph of mitochondria is reproduced in Figure 4. The mitochondrion is surrounded by a double membrane structure. Double membrane structures are also present within the interior, crossing back and forth in a step-ladder arrangement. These internal double membrane structures, or cristae, appear to be invaginations of the internal layer of the external membrane, and are arranged at right angles to the external membrane. Between these membrane structures, but sealed off from the external milieu, flows the internal fluid of the mitochondrion, in which are contained some of the essential enzymes and cofactors. If the mitochondrion is ruptured, these substances leak out, and those enzymic functions which are dependent upon them are lost. Other mitochondrial components, however, such as those which comprise the electron transport system, are firmly bound, and remain associated with the membrane structures even after rupture of the mitochondrion. There is a close correlation between the number of cristae in a mitochondrion and the functions which it performs: the more cristae the mitochondrion

FIG. 4. Electron photomicrograph of mitochondria in rod cells of the cock retina (the basic points of structural detail are essentially the same as in heart muscle mitochondria—the only reason for using this example is its exceptional clarity). Photograph taken by H. Fernandez-Moran.

contains, the more specialized it is for oxidative phosphorylation; the fewer cristae it contains, the greater is the number of its ancillary functions. The heart muscle mitochondrion has an extremely high concentration of cristae [*27*], indicating the pre-eminence of energy production among its metabolic activities (*cf.* Figure 3 for comparison of a heart muscle mitochondrion with a liver mitochondrion).

By the application of various treatments in the biochemical laboratory, isolated mitochondria may be comminuted into small particles. Mild procedures, such as a short period of sonic oscillation, yield a particle which is essentially a miniature mitochondrion [*28*]. Under other conditions, a smaller particle, known as ETP_H, can be obtained [*27,29*]. This particle has lost all resemblance to its parent mitochondrion, except for its double membrane structure, but nonetheless retains the entire electron transport system still perfectly linked to the production of ATP. (Fig. 5.) It no longer has a functioning citric acid cycle, since some of the enzymes and cofactors necessary for that process leaked out when the mitochondrion was ruptured. By a slightly different technic, mitochondria can be fragmented to yield an even smaller particle, known as the electron transport particle (ETP) [*27,30*], which has lost both the double membrane structure and the capacity to carry out oxidative phosphorylation. (Fig. 6.) The electron transport system in this particle, however, is wholly preserved. It seems, therefore, that the double membrane structure is the *sine qua non* for the coupling of electron transport to the esterification of phosphate. The tightly bound components of the non-phosphorylating electron transport particle, ETP, are arranged in polymeric sub-units of still smaller size, and isolation of this ultimate unit of the electron transport system has been approached, if not yet achieved. Recent studies with the electron microscope indicate that the monomeric, or individual unit of the electron transport system corresponds closely in size and shape with what has been suggested by biochemical evidence.

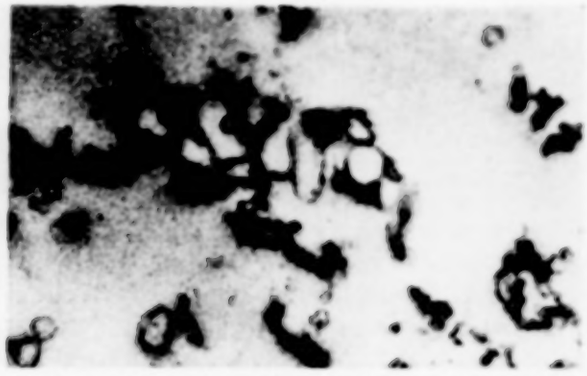


FIG. 5. Electron photomicrograph of ETP_H taken by H. Ris [*27*].

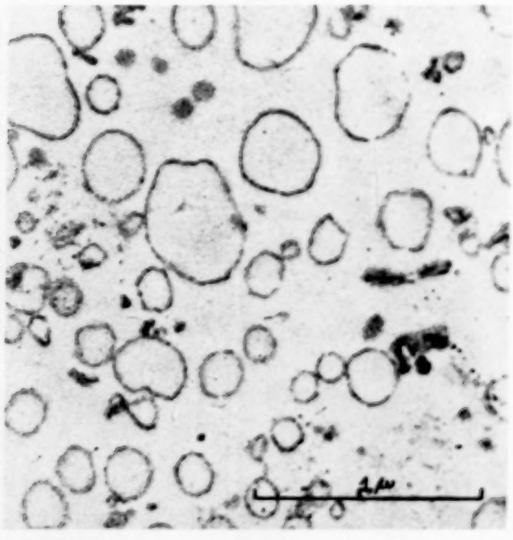


FIG. 6. Electron photomicrograph of ETP taken by H. Ris [*27*].

Electron Transport System. The known components of the electron transport system are two

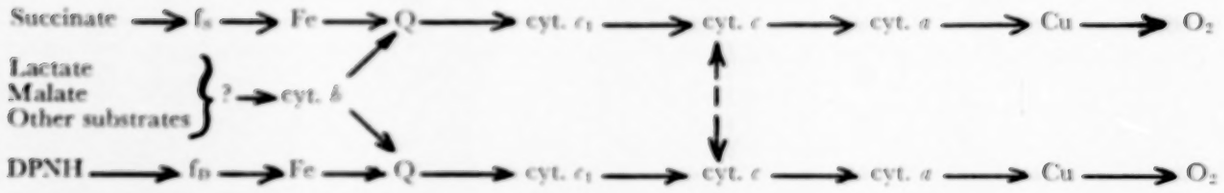


FIG. 7. Schematic representation of the electron transport system of heart mitochondria. The following abbreviations are used in this figure: f_S (succinic flavoprotein); f_D (DPNH flavoprotein); Fe (non-heme iron); cyt. (cytochrome); Cu (copper); and O_2 (oxygen).

flavoproteins [*31–33*], four cytochromes [*34–39*], coenzyme Q [*40–43*], non-heme iron [*30,44,45*] and copper [*30,44,46,47*]. Electrons derived from the oxidation of succinate enter the system by way of one of the flavoproteins (called f_S). Electrons derived from the other four oxidations of the citric acid cycle cannot enter the chain directly, but are first transferred to DPN. Reduced DPN (DPNH) can then donate its electrons to the electron transport system through the other of the two flavoproteins (called f_D). From the flavoproteins, the electrons may be passed to non-heme iron, and thence to coenzyme Q, cytochrome c_1, cytochrome *c*, cytochrome *a*, copper and, finally, to oxygen. Thus, the oxidation of one member of the citric acid cycle to form the next member in the cycle is accompanied by the reduction of oxygen to form water. Studies on heart muscle preparations, the particles of which are similar to ETP, suggest that cytochrome *b* is not an obligatory link in the electron transport system from succinate or DPNH to oxygen [*48*]. Recent work at the Enzyme Institute indicates that it may, however, serve as an alternative entry point into the electron transport system for substrates other than succinate and DPNH. It can be shown, for instance, that heart mitochondria contain an enzyme that catalyzes the oxidation of malate (in the absence of DPN), with concomitant reduction of cytochrome *b* [*49*]. It will be interesting to discover whether the same phenomenon holds true for lactate; such an observation might hold the key to the dilemma of how the heart is able to use lactate as a major energy source. In addition, it may be through cytochrome *b* that the electrons from ETF (*cf.*, fatty acid oxidation) are fed into the electron transport system. Non-heme iron is the most recently discovered member of the electron transport system [*45*]. This oxidation-reduction component appears to be situated between the flavoproteins and coenzyme Q. The available evidence at the present time indicates that there are two electron transport chains; one for succinate, the other for DPNH. The two chains must be functionally interconnected, since either succinate or DPNH can reduce all of the components of both chains. A schematic representation of the electron transport system is outlined in Figure 7.

Several individual components of the electron transport system have been isolated in pure form. Among these may be listed cytochrome *b* [*34*], coenzyme Q [*40*], cytochrome c_1 [*50*] and cytochrome *c* [*51*]. However, because a highly organized structural arrangement is necessary for the functioning of the electron transport system, the process cannot be studied adequately by merely mixing isolated components in a test tube. A more fruitful approach has been the fragmentation of mitochondria into particles which represent only a part of the electron transport system, yet retain the structural configuration necessary for the functioning of that part. By the use of various reagents, mitochondria have been fragmented to yield such particles, which are far smaller than ETP or ETP_H. A list of some of these particles, showing their composition and catalytic activities, is presented in Table IV. By correlating the components of each of these particles with the enzymic activity of the particle as a whole, it is possible to discover a great deal about the sequence and mechanism of reactions of the electron transport systems.

Another way in which the sequence of electron transport may be deduced is from the study of specific inhibitors. Antimycin A is one such compound, which inhibits the reduction of cytochrome c_1, cytochrome *c*, cytochrome *a* and copper, while it inhibits the oxidation of the other members of the electron transport system [*56*]. This observation indicates that cytochrome c_1, cytochrome *c*, cytochrome *a* and copper lie on the oxygen side of the inhibited site, whereas the

TABLE IV

MITOCHONDRIAL PARTICLES WITH ONLY PARTIAL ENZYMIC ACTIVITIES

Mitochondrial Fragment	Name of Enzyme Complex	Enzymic Activity		
		Electron Donor (substrate oxidized)	Final Electron Acceptor (substance reduced)	Ref No.
f_S, Fe*	Succinic dehydrogenase	Succinate	Various artificial acceptors (dyes)	32
f_D, Fe	DPNH-dehydrogenase	DPNH	Various artificial acceptors (dyes)	31
f_S, Fe, cyt. *b*	Succinic-coenzyme Q reductase	Succinate	Coenzyme Q	52
f_S, Fe, Q, cyt. *b*, cyt. c_1	Succinic-cytochrome *c* reductase	Succinate	Cytochrome *c*	53
f_D, Fe, Q, cyt. *b*, cyt. c_1	DPNH-cytochrome *c* reductase	DPNH	Cytochrome *c*	54
cyt. c_1, cyt. *c*, cyt. *a*, Cu	Coenzyme Q oxidase	Coenzyme Q	Oxygen	55
cyt. *a*, Cu	Cytochrome *c* oxidase (cytochrome oxidase)	Cytochrome *c*	Oxygen	47

* The following abbreviations are used in this table: Fe (non-heme iron); Q (coenzyme Q); cyt. (cytochrome); and Cu (copper).

other electron transport components lie on the substrate side.

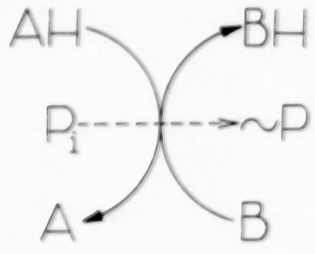
Aside from the components of the electron transport system which have been mentioned, and to which we assign specific functions, there are two great masses of material which are also necessary to the functioning of the system as a whole. They are protein (other than that which makes up part of the specific components already discussed) and lipid. The protein which forms part of the oxidation-reduction components of the electron transport system accounts for only about 30 per cent of the total protein of ETP, leaving about 70 per cent of the protein unaccounted for. For a long time this large amount of protein was ignored. Recent studies at the Enzyme Institute indicate that it has an important function in the mitochondrion [*57*]. The available evidence suggests that a portion of this protein forms the structural backbone for the electron transport system. It appears to be an organized superstructure, built up from small units to form a large polymer. The polymerization of the small units imparts to the structural protein its insolubility, which is necessary if the mitochondrion is to be preserved from dissolving in the cytoplasm of the cell. It also may provide a means for complexing the oxidation-reduction components of the electron transport system in an arrangement which would permit their interlocking functions to take place. The electron transport system may thus be visualized as a three-dimensional mosaic, the oxidation-reduction components representing tiles positioned within a matrix of structural protein.

Another substance of great importance in the mitochondrion is lipid. More than 30 per cent of the weight of the mitochondrion is contributed by lipid [*58*]. The significance of this large amount of lipid was not appreciated until recently. The evidence now indicates that lipid plays a key role in the processes of electron transport and oxidative phosphorylation [*59–62*]. Lipid has been found to be associated with f_D [*31*], $f_S b$ [*52*], cytochrome c_1 [*35*], cytochrome *c* [*63,64*] and coenzyme Q, the last of which is itself lipid soluble. In addition, several of the catalytic activities of the electron transport system of mitochondria and ETP are inactivated by extrac-

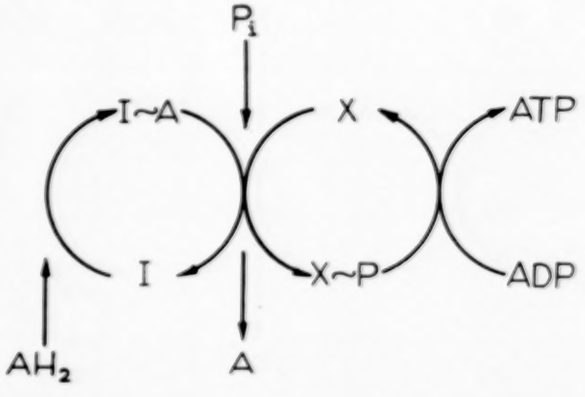
tion of the lipid from these particles and restored by adding back mitochondrial lipid [*65,66*]. It seems clear, then, that lipid performs an essential function in the electron transport process. It may provide the proper medium in which the smaller components of the electron transport system (such as coenzyme Q, non-heme iron and cytochrome *c*) can shuttle electrons between the larger members which may be more rigidly fixed to the structural protein. The importance of lipid for the process of oxidative phosphorylation may be to provide segments of non-aqueous medium in which the high energy intermediates are formed and remain stable before transfer of their energy into the bond energy of ATP.

Oxidative Phosphorylation. The process of oxidative phosphorylation was discovered in 1939 [*67–69*] but it was not until ten years later that an integrated concept was formulated to relate this process to those of the citric acid cycle and electron transport [*5*]. Oxidative phosphorylation is the linking of phosphate esterification to the oxido-reductions that occur as electrons flow through the mitochondrial electron transport system. In the transfer of electrons from one mole of DPNH to oxygen, about 52,000 calories are released; from succinate to oxygen, about 37,000 calories. However, not all of that energy is captured in the bond energy of ATP. The formation of one mole of ATP from ADP and inorganic phosphate takes up about 7,700 calories. Since three molecules of ATP are formed for each DPNH molecule oxidized (two molecules of ATP for each succinate molecule oxidized), the energy converted to the chemical bond energy of ATP for one mole of DPNH is about 23,000 calories; for one mole of succinate, about 15,500 calories. The rest of the energy released in the oxido-reductions of the electron transport system is lost and dissipated as heat. The efficiency, in terms of energy conservation, of the coupling of electron transport to the esterification of phosphate, is about 44 per cent when DPNH is used as the substrate, and about 42 per cent when succinate is used [*70*].*

In its simplest terms, oxidative phosphorylation may be represented as follows:



where A and B represent two consecutive members of the electron transport system. This formula indicates that at any given phosphorylation site, as one reduced member of the electron transport chain is oxidized by the next member of the chain, some of the energy released is captured in a chemical bond (represented by a squiggle) by the esterification of inorganic phosphate (Pi). There is no direct proof that the primary high energy bond formed in this process is a phosphate bond. The possibility of other high energy intermediates, such as a sulfur compound, as the primary traps for the energy released in the oxido-reduction must be considered. In any case, the primary acceptor is only an intermediate in the formation of ATP. Recent evidence [*71*] points to the probability of a sequence of reactions through which the high energy bond is passed until it reaches ADP, where the formation of ATP is finally accomplished. This process may be represented diagrammatically as follows:



where A is a member of the electron transport system (as in the previous formula), and I and X form hypothetical intermediates in the transfer to ATP of energy released by the oxidation of AH_2. The term I is introduced to indicate that the primary high energy bond that is formed

* The value for the free energy of ATP hydrolysis is not altogether certain. If the older figure of about 11,000 calories is used instead of the one given herein (about 8,000 calories), calculation of the efficiency of energy conservation in the process of oxidative phosphorylation would give 58 per cent for succinate and 60 per cent for DPNH.

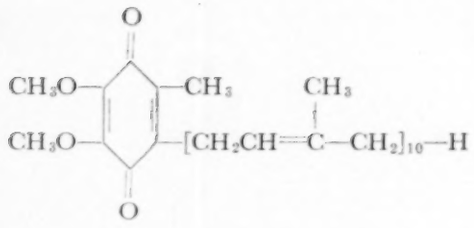


FIG. 8. Structure of coenzyme Q.

need not necessarily involve the participation of phosphate.

The specific sites at which passage of electrons from one member of the electron transport chain to the next is accompanied by phosphate esterification are not yet clearly defined. Experimental evidence obtained over the last ten years [*72–78*] has implicated three general segments of the chain as phosphorylating sites: (1) the oxidation of DPNH by flavoprotein; (2) the oxidation of flavoprotein by cytochrome *c*; and (3) the oxidation of cytochrome *c* by oxygen. Reference to Figure 7 will disclose that these "sites" are actually whole segments of the electron transport chain. Before the exact mechanism of oxidative phosphorylation can be worked out, the specific oxido-reductions which are involved in phosphate esterification must be identified. Certain members of the electron transport system were discovered only recently, and these new components may play key roles in oxidative phosphorylation. Coenzyme Q was found in beef heart mitochondria by Crane et al. at the Enzyme Institute in 1957 [*40*]. Its structure, which was worked out by the collaboration of workers from the Enzyme Institute and from the Merck, Sharp and Dohme Research Laboratories [*79,80*], is shown in Figure 8. Coenzyme Q is an extremely small molecule, compared to the other components of the electron transport chain, and the long isoprenoid side chain makes it soluble in lipid solvents. The ease with which such a small molecule could move in the lipid areas of the mitochondrion has led to much speculation as to whether it is also directly involved in oxidative phosphorylation. The quinone structure allows for the possibility of reduction to the monophosphoric ester, which might conceivably be the all-important primary high energy intermediate.

Through the investigation of such problems as electron transport a new concept in biochemical thinking has emerged: organized enzyme systems. A prime example of this concept is found in the mitochondrion, where consideration of individual enzymic activities has little meaning outside the context of their organization. Here function and structure take on significance only in relation to one another. This broader view in enzyme chemistry has yielded exciting glimpses of the future: the possibility of bridging the gap between biochemistry and physiology. It is only in the fulfillment of this possibility that cardiac metabolism can have meaning in terms of cardiac function.

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Metabolic Activity of the Intact Heart*

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THE difference in composition of blood collected from an artery and from the coronary sinus is determined by the processes of intermediary metabolism in the heart muscle cell; but, because of storage of substrates and their interchange in heart muscle, the coronary arteriovenous difference reflects only a balance and permits no conclusions as to the pathways of intermediary metabolism in the heart muscle cell. The limitations of such biochemical observations are exemplified by studies on the nitrogen equilibrium of the body. This furnishes information on the balance between dietary protein and its relation to protein breakdown or protein synthesis; but such studies tell nothing of the rate and degree of deamination, transamination, decarboxylation, or other intermediary processes of protein metabolism. It is therefore essential to combine metabolism balance studies with investigations of cellular metabolism. Although this report is primarily concerned with *in vivo* studies, using intubation of the coronary sinus, frequent reference will be made to results pertaining to the intermediary metabolism of heart muscle.

METABOLISM OF THE NORMAL HEART

Carbohydrate Metabolism. The normal human heart and the dog heart utilize glucose, pyruvate, and lactate [*1,2*]. In the human heart, both myocardial glucose usage and extraction are functions of the arterial glucose concentration. At blood sugar concentrations below 80 mg. per cent, the coronary arteriovenous glucose difference is less than 4 mg. per cent. As the arterial blood glucose concentration increases, the myocardial extraction rises rapidly; at blood concentrations above 100 mg. per cent the extraction of glucose reaches its maximum value. As in the case of glucose, myocardial utilization of lactate is dependent on its concentration in arterial blood. At normal concentrations, glucose and lactate are used by the human heart in approximately equal amounts [*1,2*]. Pyruvate is utilized by the human heart, but the blood concentration is low and so is the myocardial utilization of this metabolite. Apparently, if complete oxidation of carbohydrates is assumed, the total aerobic metabolism in man and dog accounts for only approximately 35 per cent of the total myocardial oxygen extraction, suggesting that the heart also utilizes non-carbohydrate material as fuel [*1,2*].

The myocardial utilization of fructose has recently been investigated [*3*]. It is known that fructose is rapidly removed from the blood stream after intravenous injection [*4*]. This removal is unimpaired or only slightly impaired in diabetes [*5,6*]. Using coronary sinus catheterization, no consistently high arterial-coronary sinus differences of fructose across the normally functioning myocardium have been found [*3*]. Although muscle tissue contains fructokinase, there is little utilization of fructose in eviscerated animals [*7,8*]. However, the isolated rat diaphragm can metabolize fructose [*9,10*]. Utilization is accomplished partly by non-specific hexokinase, and partly by fructokinase [*10*]. When both fructose and glucose are presented to the rat diaphragm in equal quantities, the muscle uses glucose preferentially, both for glycogen formation and for oxidation of carbon dioxide [*11*]. In addition, Hers [*12*] has shown, using C^{14}-labeled fructose, that little if any muscle glycogen is derived from direct utilization of fructose. Finally, in man, almost all of the injected fructose can be accounted for without postulating utilization outside of the splanchnic bed [*13*]. It appears, therefore, that fructose is not actively utilized by the myocardium. In several patients and dogs studied in this laboratory, considerable negative myocardial balances of fructose were observed [*3*]. This could have resulted from diffusion of

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fructose from the heart muscle into the coronary vein blood. Weichselbaum and co-workers [*14*] found negative arteriovenous differences across the human extremities following the termination of intravenous fructose infusion. These differences presumably were obtained during falling arterial blood levels of fructose, and were interpreted as back diffusion of fructose from the interstitial fluid, the intracellular fluid, or both. The quantity of fructose found in biopsy specimens of both muscle and skin suggested that free fructose was present both in the interstitial and intracellular fluid [*14*]. The possible mechanism of this back movement of fructose from the intracellular compartment may lie in changes in equilibrium between sugars that can and cannot be metabolized. The heart has at least two compartments available to act as a reservoir for fructose. The first is the interstitial fluid, which is in equilibrium with the plasma fructose. During rapidly falling blood levels this equilibrium would shift in favor of net movement into the plasma. The second compartment available is the myocardial intracellular fluid. The supposition that this fluid can act as an additional fructose reservoir is not inconsistent with current concepts of sugar transport across the cell membrane. On the basis of these considerations the positive myocardial balance of fructose described may be as well explained by net movement out of the intravascular compartment into the interstitial and intracellular spaces [*3*].

Fatty Acid Metabolism. Utilization of fatty acids by the heart was demonstrated by means of coronary sinus catheterization in 1954 when it could be shown that in the postabsorptive state about three-fifths of the energy expenditure of the heart was sustained by fatty acids [*15*]. Gordon [*16*] and also Dole [*17*] demonstrated that the principal fraction of plasma concerned with the transport and metabolism of fatty acids is the plasma non-esterified or free fatty acid (FFA) fraction. Gordon [*16*] demonstrated that the heart uses a considerable amount of FFA. It was also shown that when the fatty acid levels in blood were increased by high fat intake, the myocardial extraction ratio of fatty acids was frequently above 100 per cent; at that time two possible explanations for the high fatty acid uptake by the myocardium were suggested: that the metabolism of fatty acids was increased and that storage of fat in the heart muscle had occurred [*15*]. Although the possibility of storage occurring under these conditions remains a good one, transport of fat from the heart through lymphatic channels should also be considered.

Further investigations of the role of fatty acids in myocardial metabolism revealed that in the fasting human subject the mean myocardial extraction of free fatty acids accounts for 42 per cent of the total fatty acid extraction, the esterified fraction making up the other 58 per cent [*18*]. In the fasting dog the free fatty acid fraction accounts for only 23 per cent of the total fatty acids extracted. Gordon and Cherkes [*16*], and also Olson [*19*], have calculated that the heart can extract fairly large amounts of free fatty acids. Results in this laboratory have confirmed this, but have demonstrated that even in the fasting state free fatty acids account for less than half of the myocardial extraction of total fatty acids [*18*]. The likelihood exists that a large fraction of total fatty acids removed by the heart is not immediately oxidized to carbon dioxide and water. The large myocardial extraction of esterified fatty acids is consistent with the finding that more than half of the chylomicron triglycerides are directly oxidized [*20*]. These substances may be removed directly by the same tissue sites that oxidize plasma FFA; lipoprotein lipase present within or on the surface of the myocardial cell may hydrolyze the triglycerides at the site of their oxidation [*20*]. On the basis of the work of Fredrickson and Gordon [*20*], it is unlikely that phospholipids or cholesterol esters are metabolized by the heart.

In a recent study by Rothlin [*21*] the myocardial extraction of individual free fatty acids was investigated. Since it was found in the course of this investigation that the rate of myocardial uptake of individual free fatty acids differed, the work was extended to include the release of individual free fatty acids by adipose tissues as well. Using gas chromatography, significant differences were found between the free fatty acid composition of arterial and coronary venous blood. The proportion of oleic acid in coronary venous blood was less than that in arterial blood, demonstrating a high degree of extraction. In both man and dog the percentage of palmitic acid in coronary sinus blood was higher than that in arterial blood, and in the dog the proportion of stearic acid also was higher in coronary sinus blood, indicating a low rate of myocardial uptake [*21*]. Apparently, although the human and dog heart extract all individual free fatty acids, there are significant differences in the rate

of their myocardial uptake. This was indicated by the relatively larger percentage of myocardial extraction of oleic acid. In addition to its increased uptake by the myocardium, oleic acid was also released to a larger extent by subcutaneous adipose tissue. Apparently, the various free fatty acids appear to possess different turnover rates. However, definitive proof of this is still lacking, since release of free fatty acids and their uptake has been studied only in subcutaneous tissue and the heart [*21*].

As outlined in a preceding paragraph, coronary arteriovenous differences are determined not only by direct utilization of certain substrates, but also by interchange within the metabolic pool. These possibilities must be kept in mind when the arteriovenous differences of individual free fatty acids are interpreted. In the first place, preferential uptake of oleic acid by the heart muscle cell is a possibility. However, observations by Neptune [*22*] on striated muscle incubated in a medium containing different C^{14}-labeled free fatty acids make this unlikely. This investigator measured the uptake and oxidation of free oleic acid and found that it was not any higher than that of other free fatty acids. Conversion of oleic acid to stearic acid by hydrogenation must also be considered. This is unlikely since it has been shown that hydrogenation of oleic acid appears not to take place in the animal organism [*23*]. Finally, triglyceride fatty acids may be hydrolyzed to free fatty acids in the presence of lipoprotein lipase. Borgstrom [*24*] has demonstrated an exchange between triglyceride fatty acids and free fatty acids in the presence of this enzyme. Consistent with this concept are observations by Havel [*25*] who showed that C^{14}-labeled triglyceride acids when injected intravenously reappear in the plasma FFA fraction.

Considering the high myocardial uptake of esterified fatty acids, and the large amount of lipoprotein lipase present in the heart muscle, the likelihood of exchange between triglycerides and free fatty acids in heart muscle is great. As a result, the free fatty acids in coronary sinus blood originating partly from hydrolysis of triglycerides through the action of lipoprotein lipase may have a different composition from the free fatty acids in arterial blood. The observations of Feldman [*26*] that lipoprotein lipase activity is inversely proportional to the chain length and degree of unsaturation of these fatty acids also supports this hypothesis. Accordingly, hydrolysis of the more saturated fatty acids, such as palmitic and stearic acids, should progress at a higher rate than that of unsaturated fatty acids, such as oleic and linoleic acids. This is in accord with the observation presented.

Myocardial utilization of ketone bodies and amino acids has been reported [*15*]. The aerobic metabolism of ketones accounts for approximately 5 per cent of the total oxygen extraction [*15*]. In diabetes, the contribution of ketones to the oxidative metabolism of the heart is much greater [*27*]. It is likely that ketone utilization by the myocardium is governed by the arterial concentration and by the quantity of carbohydrates available. Amino acids also are extracted by the heart muscle [*15*]. After infusion of amino acids as much as 40 per cent of the total cardiac oxygen consumption can be accounted for by aerobic metabolism of amino acids [*15*]. A small rise (20 per cent) in arterial blood amino acid content produces a disproportionate increase (245 per cent) in myocardial extraction of amino acids [*15*]. Investigation of the uptake of individual amino acids by the heart would be of interest. However, here too, as in the case of fatty acids, conclusions as to the fate of individual amino acids in heart muscle are difficult to arrive at because of their transformation into other nitrogenous compounds. It is likely that the myocardial balance of amino acids is also influenced by continuous exchange between exogenous and endogenous amino acids, both of which can be used for protein synthesis.

METABOLISM OF THE ANOXIC HEART

A number of pathologic states may induce anoxia in cardiac muscle, with a consequent shift of cardiac metabolism toward anaerobiosis.

Myocardial anoxia is accompanied by a negative balance of lactate and frequently of pyruvate. This has been observed in hemorrhagic shock, ventricular tachycardia and fibrillation, atrial fibrillation, and following temporary interruption of the coronary flow after embolization of the coronary arteries [*28–31*]. The cellular mechanisms underlying this reversal in the myocardial balance of lactate and pyruvate have been studied with particular reference to the metabolism of intermediates of the glycolytic cycle and to phosphorylase activity. Anoxia of the heart muscle, regardless of the mechanism of its production, leads to a disappearance of glycogen and to an increase in lactate and glucose-6-phosphate in heart muscle,

suggesting that under these conditions phosphofructokinase activity becomes the rate-limiting step [*32*]. In addition, myocardial anoxia as it occurs in ventricular tachycardia and fibrillation leads to a transitory increase in the ratio phosphorylase a : total phosphorylase [*30*]. This is in accord with Cori's [*33*] finding in skeletal muscle, that when the gastrocnemius muscle of the rat was stimulated at a high rate, there regularly occurred an increase in the level of active phosphorylase. A fall in oxygen tension in heart muscle must necessarily be accompanied by a shift in the oxidation-reduction systems toward a more reduced state. The extent to which individual members of the electron carrier systems shift toward the reduced form is determined by the inherent redox potential of the system in a manner reminiscent of the way the pK of a buffered system governs shift in pH [*34*]. Diphosphopyridinenucleotide (DPN) has the lowest potential in the carrier system. As Huckabee [*35*] has pointed out, the rates of oxidation for energy production are not altered until DPN has been affected. When the oxidative potential has become low enough metabolic systems are involved; the first DPN coupled system to be reversed will be the one with a potential closest to that of DPN^+ to DPNH, or the lactic dehydrogenase system: Pyruvate + DPNH $\overset{LDH}{\rightleftharpoons}$ Lactate + DPN^+. An increase of DPNH would (by mass action) shift the equation to the right, with production of lactate and oxidation of DPN. Because lactate does not take part in any other equilibrium, this reaction acts as a safety valve in the presence of hypoxia, thereby permitting other metabolic oxidative systems to continue to function. Huckabee [*35*] has demonstrated the correspondence of the magnitude of excess lactate production and oxygen debt in the whole body. If, despite hypoxia and relatively high concentration of $DPNH_2$, the reaction continues to the left of the equation because of excess lactate or because of diminished production or rapid removal of pyruvate, one might expect lactate utilization to be even more reduced. Such has been found to be the case.

The heart muscle has a high degree of resistance to anoxia. It has been shown, for example, that rapid resynthesis of glycogen and ATP can take place in heart muscle during reperfusion of the coronaries after a brief period of anoxia [*32*]. The amount of glycogen which disappears anaerobically in heart muscle can be grossly accounted for as the sum of hexosemonophosphate (HMP) and lactic acid, indicating that other than these metabolites no other glycolytic intermediates accumulate in the anoxic heart [*32*]. This assumption is corroborated by the low tissue concentrations of fructose-1,6-diphosphate and dihydroxyacetone-phosphate which are found in the anoxic heart. These facts suggest that the rate at which glycogen is broken down to HMP in the anoxic heart exceeds the rate at which lactate is formed from HMP. Increased substrate saturation of phosphofructokinase leads in turn to an increase in the formation of lactic acid, as indicated by its accumulation in the muscle. However, despite the fact that substrate saturation is favored under anoxic conditions, phosphofructokinase remains the rate-limiting enzyme in the glycolytic system. Muscle contraction, under anaerobic conditions, as shown by Cori [*33*], exhibits essentially the same metabolic pattern.

It is likely, from the studies of Lorcher [*36*], that in anoxia fatty acids continue to contribute to the energy requirements of heart muscle.

Ventricular Tachycardia and Fibrillation. In these conditions the concentration of lactate and pyruvate in coronary sinus blood increases [*37,38*]. In ventricular tachycardia and fibrillation the activity of malic acid dehydrogenase in coronary sinus blood also is increased [*30*]. Ventricular tachycardia and fibrillation are accompanied by a diminution in cardiac glycogen, a slight rise in glucose-6-phosphate, a marked elevation in lactic acid, and a temporary increase in phosphorylase a activity [*30*]. When ventricular and atrial fibrillation and ventricular tachycardia are produced in hearts in which the coronary circulation is maintained, no alterations in carbohydrate intermediates or in phosphorylase a activity are observed [*30*]. Apparently, increased heart rate in the absence of anoxia fails to evoke the classic pattern resulting from increased rate of stimulation described as typical for skeletal muscle [*33*].

Hemorrhagic Shock. The changes in myocardial metabolism in shock are primarily the result of myocardial anoxia. This was suggested by Wiggers [*39*] on the basis of pressure and volume curves obtained from dogs during various phases of hemorrhagic shock; he and his co-workers suggested that the deterioration of myocardial expulsive power resulting from anoxia may contribute to circulatory failure and that this myo-

cardial depression is responsible for the irreversible shock during the normovolemic phase of hemorrhagic shock. It was assumed by Wiggers that the trigger mechanism for myocardial depression was a decrease in coronary flow. This has been confirmed by studies employing coronary sinus catheterization [*29*]. During hemorrhagic shock in dogs, coronary blood flow diminished during the oligemic and normovolemic phases. As a result of failure of oxygen extraction to increase, myocardial oxygen usage declined. In the hypovolemic phase of shock, blood levels of glucose rose to high peaks, but the rise in glucose extraction was statistically not significant. Myocardial pyruvate extraction also declined and coronary arteriovenous differences frequently were negative. Blood lactate rose to very high levels, presumably as a result of anaerobic glycolysis elsewhere in the body. Total myocardial extraction of lactate was actually higher than during control periods, but not as high as one would have expected in a fully oxygenated heart. After retransfusion to normal blood volume, the myocardial extraction of lactate and pyruvate remained diminished, while blood glucose levels fell to normal with increases in the blood volume. Myocardial glucose extraction was statistically not different from that during the control period; however, in several instances concentrations of glucose in coronary veins exceeded those in arterial blood [*29*]. Thus the essential changes in coronary blood flow and myocardial metabolism in hemorrhagic shock persisted during the normovolemic phase. In both stages, myocardial oxygen usage was low and the extraction of glucose, pyruvate, and lactate was impaired. The changes in myocardial metabolism during hemorrhagic shock are therefore not isolated occurrences but follow the general pattern of myocardial anoxia resulting from diminished coronary blood flow.

Apparently, the heart participates in the general pattern of tissue anoxia such as occurs in hemorrhagic shock. The question has been asked whether the irreversibility of shock is related to or perhaps even caused by permanent myocardial changes in heart muscle. It does appear likely that myocardial anoxia produces irreversible changes in shock, such as a fall in ATP, in phosphocreatine, and in glycogen; however, these metabolic events occur under the influence of anoxia in all metabolizing tissues of the body. The irreversibility of shock, therefore, is only the dynamic expression of irreparable damage to metabolic processes produced by prolonged tissue anoxia.

Coronary Embolization and Myocardial Infarction. Studies on circulatory changes in acute myocardial infarction in man confirmed that the primary alterations are a decrease in cardiac output, with a compensatory increase in peripheral vascular resistance. The pattern, however, is not uniform, as some patients show no increase in peripheral resistance and only little diminution in stroke volume. Wiggers and his coworkers [*40*] introduced the concept that compensation of the myocardium following coronary artery occlusion is due to enhanced action of the uninvolved portion of the myocardium rather than to improvement of the circulation through the injured area. Failure of the uninvolved myocardium to respond to this burden results in circulatory failure. There is little doubt that anoxia of the myocardium is the cause of the biochemical, histological, and dynamic changes found in coronary embolization in myocardial infarction. The technic of Agress et al. [*41*] in which plastic spheres of 325 μ diameter are injected into the coronary arteries of closed-chest dogs has made possible accurate evaluation of the sequence of events occurring in the development of myocardial infarction and necrosis. Animals in which the coronary arteries were injected with microspheres suspended in dog plasma showed a significant decrease in myocardial oxygen extraction [*31,42*]. Myocardial oxygen usage also decreased. Twenty-four hours after coronary artery embolization, all values had returned to control levels. The metabolic changes consisted in significant diminution in myocardial extraction of pyruvate and lactate. These changes, however, were only brief since in most instances all values had returned to normal after thirty minutes [*31,42*]. In contrast the myocardial usage of glucose remained unaltered, suggesting that in the short period following embolization of the coronary arteries, glucose may have been the primary metabolite. Apparently, anaerobic glycolysis can still proceed in heart muscle under these conditions. While these rapidly irreversible circulatory and metabolic changes are present only during the period of cardiac depression, the changes in plasma enzyme activity are associated with pathologic events that take place many hours following coronary arterial embolization; that is, during

development of the infarct. Thus the peak of plasma enzyme activity in the dog occurs twenty-four hours after embolization and is correlated with the height of coagulation necrosis of the myocardium. Enzymes released into the blood from the injured tissues decrease as regenerative processes take place.

The release of enzymes such as transaminase, aldolase, phosphohexoisomerase, and malic acid dehydrogenase from the heart is usually related to anoxia, since in this condition permeability of the cell membrane is increased. It is not yet clear whether the increased serum levels of these enzymes are due wholly to the release of intracellular enzymes. As an alternative explanation, a decrease in the body's degradation or excretory mechanism of these enzymes has been postulated; stimulation of increased production or release of enzymes by other non-necrotic tissue has also been considered [*43*]. The mechanism for elimination and degradation of these enzymes is highly effective. For example, Fleisher and Wakim [*44*] gave massive amounts of glutamic oxalacetic acid transaminase intravenously to dogs, causing an increase in serum activity some 212 times the value before the injection. In twenty-four hours 97 per cent of the enzyme activity had disappeared, and the control level was reached by the end of the third day. According to Hess [*45*], the cause of increase in enzymes following myocardial infarction is increased permeability of the cell membrane, resulting from deficient cellular respiration.

In general, a variety of factors which change membrane permeability can lead to efflux of cellular enzymes; among them are anoxia, lack of glucose, and exposure to iodoacetic acid, dinitrophenol, or cyanide [*46*]. Zierler [*46*] mentions that under appropriate conditions insulin also increases permeability to aldolase. The efflux of enzymes from rapidly contracting heart muscle is in all likelihood the result of the myocardial anoxia which occurs under these conditions. Visible evidence of alterations in membrane functions can be obtained with microelectrode technics. For example, Webb and Hollander [*47*] determined the membrane potential of isolated, electrically stimulated anoxic rat atria and observed that the height of the action potential fell after four minutes of anoxia. The effects of complete ischemia on the spontaneous ventricular action and resting potentials are illustrated by rapid shortening of the action potential [*48*]. After twenty minutes of complete ischemia, all spontaneous mechanical and electrical activity had ceased [*48*]. This shortened duration of the action potential is due to abbreviation of the repolarization phase. It is possible that the alterations of the cell membrane induced by anoxia are closely related to the concentration of high energy phosphate. Thus Ling and Gerard [*49*], using anoxic single frog sartorius fibers, showed a parallel fall in phosphocreatine and membrane potential during a three-hour period of observation. As a result of these changes in permeability, soluble proteins and enzymes and substances of low molecular weight, such as nucleotides, pass through the membrane and as a consequence the cell dies.

In reviewing the circulatory, pathologic, and biochemical findings following experimental embolization of the coronary arteries it appears that the factor which initiates the sequence of events in myocardial ischemia and anoxia is produced by mechanical blockage of some of the coronary vessels. The localized response to this injury appears to be coronary vasodilatation as a result of an intercoronary reflex and accumulation of anaerobic metabolites. Localized myocardial ischemia results in a period of decompensation of the affected heart muscle, with a decrease in cardiac output and blood pressure, and in immediate although fleeting changes in utilization of substrates by the heart [*42*].

Hypothermia and Cardiac Arrest. Within the last ten years cardiac metabolism has become of interest to the surgeon because of the use of cardiac arrest and of hypothermia. In principle, the problems encountered are those pertaining to myocardial anoxia. Cardiac metabolism has been studied in hearts arrested with potassium chloride, potassium citrate, and with acetylcholine. However, since these studies were based chiefly on the use of biopsies, they are not within the scope of this discussion. Mention may be made of the finding that in potassium citrate arrest of the heart, the ATP, phosphocreatine, and glycogen levels fall much more rapidly than in selective hypothermic arrest. Also, the tissue lactic acid rises more rapidly with potassium citrate arrest. Apparently, for cardiac arrest, selective hypothermia is far superior to potassium citrate arrest in maintenance of the myocardial energy resources [*50*]. The effect of ischemia and reoxygenation on glycolytic reactions and ATP in heart muscle was also extensively investigated by Danforth and his co-workers [*32*]. They

found that the concentration of glucose-6-phosphate always fell during reperfusion with oxygenated blood, rapidly reaching levels near normal. When the heart was arrested for fifteen minutes with potassium chloride, and then reperfused with oxygenated blood, ATP was rapidly resynthesized. The concentration reached initial levels in four to ten minutes [*32*]. This indicates that there was a sufficient supply of adenosine nucleotides in heart muscle after fifteen minutes of anoxia.

Using coronary sinus catheterization, the effect of hypothermia on cardiac metabolism was studied [*50*]. In these animals body temperature was reduced to 26°c. Arteriovenous oxygen differences remained unchanged from the normothermic state, but coronary blood flow and myocardial oxygen consumption decreased. Hypothermia therefore results in myocardial anoxia. In contrast to anoxia of similar degree produced in the normothermic animal, the myocardial extraction of pyruvate and lactate remains positive, illustrating the protective influence of hypothermia. It appears that herein lies the fundamental advantage of lowering the body temperature in cardiac surgery.

METABOLISM IN THE DIABETIC HEART

Coronary sinus catheterization has revealed a series of metabolic alterations to occur in the heart of patients with diabetes mellitus and of dogs with alloxan diabetes [*27,51*]. In both patients and dogs, the mean value for myocardial usage of carbohydrates is reduced and the utilization of non-carbohydrate material is increased [*27*]. Apparently, the heart is not exempt from the most important metabolic defect in diabetes, that of deficient utilization of carbohydrates [*52*]. Deficient myocardial glucose utilization has also been noted in the isolated heart *in vitro*. Evans [*53*] found that the diabetic dog heart consumed only one-fourth of the glucose utilized by the non-diabetic organ. The alloxan diabetic dog heart utilizes glucose, but in diminished amounts [*27,51*]. The myocardial lactate usage of both the diabetic human heart and diabetic dog heart also is significantly reduced. This is in contrast to results obtained by Evans et al. [*53*] who found almost unimpaired utilization of this substrate by the isolated heart. They concluded that usage of lactate replaces or supplements that of sugar in the diabetic heart. There is no evidence for this in the results obtained by coronary sinus catheterization in both the human and dog heart [*27,51*]. On the contrary, the marked reduction in myocardial usage of lactate is primarily responsible for the reduction in the total amount of energy available from the carbohydrate fraction. The deficiency in utilization of carbohydrates by the heart also extends to pyruvate [*27,51*]. Apparently, the diabetic heart extracts normal amounts of pyruvate, but this occurs in the presence of a significant elevation in the arterial concentration of pyruvate. In accord with this are the results of Pearson et al. [*54*] who found diminished pyruvate utilization in both cardiac and diaphragmatic muscle of diabetic animals. Villee, White and Hastings [*55*] also found that the rate of oxidation of acetate and pyruvate to carbon dioxide was diminished in diaphragms from alloxan diabetic rats.

In the face of reduced carbohydrate utilization, a relatively large amount of non-carbohydrate material must be used by the heart for energy production. This is indeed the case, since the human heart in patients with diabetes mellitus extracts a significantly greater amount of fatty acids than does the heart in patients without diabetes [*27,51*]. In the diabetic dog, however, despite a significant increase in the concentration of arterial blood fatty acids, myocardial extraction of fatty acids is not altered [*27*]. An increased myocardial utilization of fatty acids, such as is observed in human patients with diabetes is consistent with results obtained in the isolated heart *in vitro* [*56*]. Apparently, there is some connection between this large myocardial extraction of fatty acids and the inability of the diabetic organism to synthesize them from glucose, lactate, and pyruvate [*57*]. The usage of ketone bodies by hearts of diabetic patients is only slightly increased [*27*] but the hearts of dogs made diabetic with alloxan use a much greater quantity of ketones than do the hearts of normal animals [*27*]. The increased myocardial usage of ketones by the dog heart occurs in the presence of elevated arterial ketone concentrations. In these animals the contribution of ketones to the aerobic metabolism of the diabetic dog heart is 13.5 per cent higher than that of the normal heart. It is possible that the decrease in the rate of total oxidation of carbohydrates by the heart may have been responsible for the increased myocardial usage of ketones.

If, under these conditions insulin is added to correct the metabolic defects found in the

diabetic heart, a relative increase in myocardial utilization of fatty acids and ketones should ensue. Hormone administration results in a significant fall in blood sugar, but this occurs without change in the myocardial usage and extraction of glucose [*27,51*]. This implies that insulin causes a relative increase in myocardial glucose utilization. After injections of insulin to diabetic dogs the concentration of pyruvate in coronary vein blood frequently exceeds that in arterial blood. This diminished myocardial uptake of pyruvate by the heart muscle may be an indication of increased catabolism of endogenous carbohydrates in heart muscle, initiated by a rapidly falling blood sugar level in these severely diabetic organisms. Surprising is the finding that insulin fails to correct the metabolic defect responsible for diminished myocardial lactate usage. Arterial lactate concentration rises, but myocardial extraction of lactate hardly changes. Administration of insulin also results in a fall in the blood concentrations of fatty acids, without significantly affecting their myocardial usage or extraction. It appears likely that the fall in blood concentration of fatty acids is due to decreased mobilization from fat depots [*27,51*].

The results in the diabetic heart *in situ* demonstrate a great variety of metabolic defects. The diabetic heart is deficient in glucose, pyruvate, and lactate utilization. In addition, the defect extends to the metabolism of protein and fat. Of course, the fundamental importance of diabetes in heart disease does not relate to metabolic disease of heart muscle, but to the frequency with which diabetes results in coronary vascular changes and its complications.

METABOLISM OF THE FAILING HEART

Non-metabolic Heart Failure. There is general agreement that the myocardial extraction of various substrates in congestive heart failure is normal [*19,58,59*]. This is the case in hypertensive and valvular as well as in arteriosclerotic heart disease [*19,59*]. In addition to normal myocardial usage of substrates, patients with compensated or decompensated heart disease have normal coronary blood flow and normal myocardial oxygen usage per weight of heart muscle despite increased diastolic heart size [*19,58,59*]. Since the failing heart during exercise can increase its oxygen uptake, there appears to be no impediment to the delivery of oxygen to the myocardium. Digitalis preparations also do not affect coronary blood flow or myocardial oxygen consumption in the normal or failing human heart, although they sometimes do affect potassium efflux from the heart [*60,61*]. There is no significant effect of lanatoside-C on the utilization of myocardial substrates despite improvement of the work capacity of the failing heart [*60*].

Relative lack of high energy phosphate due to rapid deterioration or inefficient formation from normal oxidation must be considered. However, no changes in the levels of ATP or creatine-phosphate in chronic congestive heart failure have been reported in dogs with induced valvular disease [*62,63*]. Furthermore, oxidative phosphorylation in mitochondria obtained from hearts of guinea pigs in chronic congestive failure is normal [*64*]. Furchgott and deGubareff [*65*] detected no significant difference in high energy compounds in hypodynamic atria which had undergone spontaneous failure, nor did recovery from failure induced by strophanthus-K alter these results.

In most instances in which disturbances in energy production were present, heart failure was produced by localized ischemia resulting from ligation of coronary arteries. For example, Lamprecht [*66*] found that under these conditions the P:O ratio of heart muscle fell from 2.75 to 1.39. It is not difficult to understand why a diminution in phosphate uptake occurred. As already indicated, anoxia results in a significant decrease in ATP and phosphocreatine. Lamprecht found that the decrease in ATP was accompanied by an increase in inorganic phosphate. However, the increase in oxygen uptake of these preparations after interruption of the coronary blood supply is difficult to understand. It is important to differentiate between failure resulting from anoxia and its resulting metabolic changes on the one hand and failure resulting from hypertensive, valvular, and longstanding arteriosclerotic heart disease on the other.

By exclusion, the evidence points therefore to the organs of energy utilization, the contractile proteins, as the site of myocardial derangement in heart failure resulting from hypertensive, arteriosclerotic, and valvular disease. Certain advances have been made within recent years to define this defect. For example, actomyosin bands prepared from failing human hearts appear to possess diminished contractility [*67*];

Olson [*68*] has also demonstrated differences in certain physical-chemical properties of myosin. However, the question how these alterations in proteins can come about without metabolic changes has remained unanswered. The hypothesis that stretch plays an important role has never been conclusively demonstrated. A change in orientation of actomyosin is also unlikely, since Olson [*19*] observed changes in myosin molecules of failing heart muscle.

Recent observations by Meerson and Zayats [*69*] have given certain clues to the possible metabolic causes of these alterations in contractile proteins of failing heart muscle. They measured the rate of protein synthesis in the heart of rabbits with experimentally produced aortic stenosis. Protein synthesis was measured by the rate of uptake of S^{35}-labeled methionine into heart muscle. Immediately following the production of aortic stenosis, the changes occurring in protein synthesis consisted in weight increase and dilatation of the heart. The rate of protein synthesis doubled and microscopic changes in heart muscle were noticeable [*69*]. Muscle glycogen and creatinephosphate diminished, while lactic acid concentration in heart muscle rose. During the second stage, referred to as stable hyperfunction, the heart rate first increased, then remained constant, and the rate of protein synthesis returned to normal [*69*]. There was hypertrophy of the muscle fibers. The myocardial concentration of phosphocreatine and glycogen was normal, but lactic acid concentrations remained elevated. During the third stage, referred to as that of cardiac decompensation, the heart weight remained constant, but there was cardiac dilatation and protein synthesis decreased. Lactic acid concentration in heart muscle increased, creatinephosphate diminished, glycogen concentration remained unchanged. Accordingly, cardiac hypertrophy produced an increased myocardial mass and an increase in sarcosomes [*69*]. Myocardial anoxia was present, as indicated by the increase in lactic acid. The authors concluded that disturbance in protein synthesis in the myocardium was an important factor in the development of myocardial failure, and that the loss of cardiac contractility is connected with a disturbance of the normal process of protein synthesis in heart muscle. The cause for diminished protein synthesis may be prolonged anoxia with reduced ATP synthesis or a deficiency of DNA, the latter brought about by relative increase in the size of cytoplasm as compared to nuclear mass.

Accordingly, diminished protein synthesis may explain the alterations in contractile proteins of heart muscle; however, it is difficult to see how accumulation of lactic acid in heart muscle could occur without a corresponding rise in concentrations in the coronary vein blood. As already mentioned, there is no evidence of anaerobiosis in hearts failing from arteriosclerotic, hypertensive, or valvular disease. The finding of normal high energy compounds in congestive heart failure also is not consistent with these data. It must be concluded, therefore, that the factors leading to changes in contractile proteins in the absence of metabolic alterations in heart muscle are still unknown.

Metabolic Heart Failure. In this group belong heart failure from anemia and anoxia, hyperthyroidism, and thiamine deficiency.

Heart failure resulting from anemia and anoxia: The metabolic effects of anoxia have been described in a previous paragraph; it was shown that a diminution occurs in glycogen and high energy phosphate, while the level of glucose-6-phosphate increases. The ratio of phosphorylase a:b probably decreases. Under these conditions phosphofructokinase becomes the rate-limiting enzyme. Even brief anoxia, such as is present during angina pectoris and localized anoxia during myocardial infarction, leads to demonstrable glycolysis, as evidenced by increased lactate levels in coronary sinus blood [*42,70*]. Consequently, cardiac failure occurring as a result of anoxia is initiated by deficiencies in energy production. Dynamically, in anemic heart failure there is increased cardiac output and tachycardia. Because of the decreased blood oxygen capacity, myocardial oxygen extraction falls. However, the coronary blood flow rises markedly, and the total myocardial oxygen consumption may be higher than in the normal person [*71*]. Experiments in the open chest dog have given similar results in respect to the coronary blood flow in response to anemia. When the hematocrit is reduced to levels of 24 to 31 per cent, there is depression of ventricular function curve [*72*]. This loss of the ability of the heart to increase its work occurs when the coronary bed approaches maximal dilatation, suggesting that despite coronary vasodilatation, delivery of oxygen to the myocardium is inadequate.

Hyperthyroidism: Early studies with coronary

sinus catheterization suggested that in hyperthyroidism the oxygen consumption of the heart is normal [*71*]; however, as more patients were studied it became apparent that the heart of both thyrotoxic man and dog share in the general increase of body metabolism, although recent observations again cast doubt on this supposition [*70,73,74*]. The coronary blood flow, the myocardial substrate utilization, and the oxygen consumption are all increased [*73*]. Myocardial oxygen utilization returns to normal following remission of thyrotoxicosis [*73*]. Normal myocardial extractions of glucose, lactate, and pyruvate have been reported in thyrotoxic patients [*75*]; in hyperthyroid dogs these extractions were diminished [*74*]. The underlying metabolic disturbance may be sought in the action of the thyroid hormone, which results in "uncoupling" of oxidative phosphorylation, leading to inefficient energy conservation; under these conditions more oxidation of substrate is required to produce the same amount of high energy phosphate. Heart mitochondria are particularly susceptible to this effect of the hormone [*76*]. It is therefore to be expected that high energy phosphate compounds in these conditions are reduced in the heart muscle. This could not be substantiated by Piatnik and Olson [*74*], but in their experiments the dogs apparently were not in heart failure; consequently, insufficient energy conservation is still a possible cause of the congestive heart failure seen in thyrotoxicosis.

Thiamine deficiency: Thiamine deficiency resulting in beriberi chiefly affects the nervous tissue, usually as Wernicke's syndrome, and the heart. Cardiac beriberi is arbitrarily divided into the acute fulminant form and the chronic type. Shoshin beriberi is the fulminant form of beriberi heart disease, which is characterized by acute cardiovascular collapse [*77*]. This form of the disease comprises less than 5 per cent of the cases of beriberi with cardiac involvement seen in the Orient. Two cases of shoshin beriberi heart disease were recently described from this institution by Wolf and Levin [*77*]. Beriberi heart disease has been produced in various laboratory animals through the use of thiamine-deficient diets [*78*], and Olson [*79*] has studied the *in vitro* metabolism of cardiac muscle obtained from rats and ducks fed such thiamine-deficient diets. He was able to show that the rate of pyruvate utilization by slices of thiamine-deficient rat and duck ventricle was significantly reduced. There was also a relationship between the rate of pyruvate disappearance and the thiamine content of the ventricle. Apparently, pyruvate utilization in these preparations did not fall until the thiamine content was reduced from a normal value of about 10 μg./gm. to about 2.5 μg./gm. Hackel and Goodale [*80*], using coronary sinus catheterization, found in dogs that the coronary arteriovenous difference and total utilization of pyruvate in thiamine-deficient dogs were maintained within normal limits. The threshold of utilization of pyruvate was significantly increased, however, and the coefficient of its extraction was diminished. Apparently, myocardial lactate extraction was inhibited more than pyruvate, possibly due to the adverse effects of elevated pyruvate levels on lactic dehydrogenase. Glucose extraction by the myocardium also was below normal in acute thiamine deficiency, but was not different from the finding in starved animals. There was only slight elevation of cardiac output and coronary blood flow and the mean arterial pressure was normal.

The observation of Hackel and his co-workers [*80*] in thiamine-deficient dogs, that at larger flow the oxygen content of coronary sinus blood was elevated to a greater degree than expected, resulting in a marked diminution of myocardial oxygen extraction, suggests that deranged metabolism may result in insufficient energy production which could well account for the decreased ATP levels observed in the hearts of thiamine-deficient rats, as demonstrated by Chen and Geiling [*81*].

In a patient with beriberi heart disease, studied by Olson and his co-workers [*79*], cardiac catheterization revealed a cardiac output of 25 L./minute and a coronary flow of over 200 cc./100 gm./minute. The myocardial extraction of pyruvate and lactate by the heart was reduced below normal. The infusion of glucose into this patient resulted in abnormal elevations of pyruvate and lactate with only small changes in extraction of these substrates by the heart, and no change in respiratory quotient [*79*].

There are several biochemical causes for these metabolic disturbances. Thiamine pyrophosphate is necessary for the opening step of the reactions which feed into the citric acid cycle [*82*] where pyruvic acid is decarboxylated and active acetaldehyde is combined transiently with the thiamine before it is transferred to the oxidized form of lipoic acid. The lipoic acid is thereby reduced and acetyl lipoic acid comes into being.

There is a second reaction, entirely analogous to that in which pyruvic acid participates. This is the oxidative decarboxylation of α-ketoglutaric acid. This acid is decarboxylated and "active" succinyl semi-aldehyde is formed momentarily in combination with thiamine, and then is transferred to lipoic acid, giving rise to succinyl lipoic acid from which the succinyl group is transferred to coenzyme A [*82*]. A third reaction in which thiamine participates has been described. This is the transketolase reaction. Thiamine pyrophosphate acts as coenzyme in this reaction, and there exists an intermediate "active glycoaldehyde" analogous to the previously mentioned "active acetaldehyde."

It is likely that in beriberi the heart participates in the general metabolic defect induced by thiamine deficiency. In contrast to heart failure resulting from arteriosclerotic, hypertensive or valvular disease, beriberi heart disease is the result of deficient energy production in the heart muscle.

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The Contractile Proteins of Heart Muscle*

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THE metabolic processes in heart muscle may be divided into three general phases. These are (1) energy liberation, (2) energy conservation, and (3) energy utilization [1]. (Fig. 1.) In the phase of energy liberation the carbon-carbon and carbon-hydrogen bond energy of substrate is liberated as free energy. Specifically, the processes of glycolysis, fatty acid and pyruvic acid oxidation, and the dehydrogenations of the Krebs tricarboxylic acid cycle occur in this phase and result in conversion of the bond energy of substrate into the free energy of hydrogen electrons, which are transported to oxygen along the respiratory chain of the sarcosome. The second phase of energy conservation includes the processes of oxidative phosphorylation by which the energy of hydrogen is converted into the terminal bond of adenosinetriphosphate (ATP) and, via creatine kinase, to creatine phosphate (CP). The third phase of energy utilization includes the mechanisms by which the terminal high energy phosphate bond of ATP is channeled into a variety of anabolic processes and into the contractile process, which results in mechanical work. In heart muscle most of the energy liberated and conserved in ATP is channeled into mechanical work. This is impressively dramatized by the observation that the oxygen consumption of the normal mammalian heart delivering a cardiac output of 1.5 L. per 100 gm. of heart muscle per minute is reduced from 10 cc. of O_2 per 100 gm. per minute to less than 1 cc. of O_2 per 100 gm. per minute by chemical arrest at 37°C. (2). Detailed considerations of the first two phases of cardiac energetics have been given by other contributors to this symposium. This paper is devoted to a discussion of the properties of the contractile proteins of striated muscle (both skeletal and cardiac) and a review of our knowledge of the biochemical events which occur in the contractile cycle.

Since the pioneer work of Hill [3] and Meyerhof [4], muscle physiologists have sought a hypothesis which would adequately explain the coupling of chemical energy to mechanical work in the myofibril. Meyerhof's view that lactic acid fermentation was the direct source of energy for contraction gave way to Lundsgaard's view [5] that creatine phosphate was the immediate source of the contractile power. Refinements in our knowledge of the formation of high energy phosphate bonds [6] during the past two decades [7] have led to the view that ATP is the immediate source of energy for contraction and that CP is utilized to replace ATP if the steady generation of ATP is retarded or blocked. Exact knowledge of the nature of the chemomechanical energy coupling remains elusive. Even more obscure is the link between membrane depolarization and initiation of contraction. The discovery of myosin by Kühne in 1864 [8] and of actin by Szent-Györgyi in 1943 [9] led to the study of contractility *in vitro*. Currently there are no less than six hypotheses regarding the contractile mechanism [10–15]. Five of them deal with the interactions of the two major contractile proteins, myosin and actin with ATP, and the sixth [10] relates this system to a third protein, tropomyosin. Although these three proteins (myosin, actin and tropomyosin) appear to be the major elements in the contractile system, with ATP the energy source, great controversy centers around the actual biochemical events in the cycle of shortening and relaxation. Most of these hypotheses have been developed from data obtained in studies of skeletal muscle, but most appear applicable to cardiac muscle as well.

The plan of this presentation will be: first, to discuss the properties of the contractile proteins which have been isolated from striated muscle, both skeletal and cardiac; second, to summarize the evidence which is available as to the bio-

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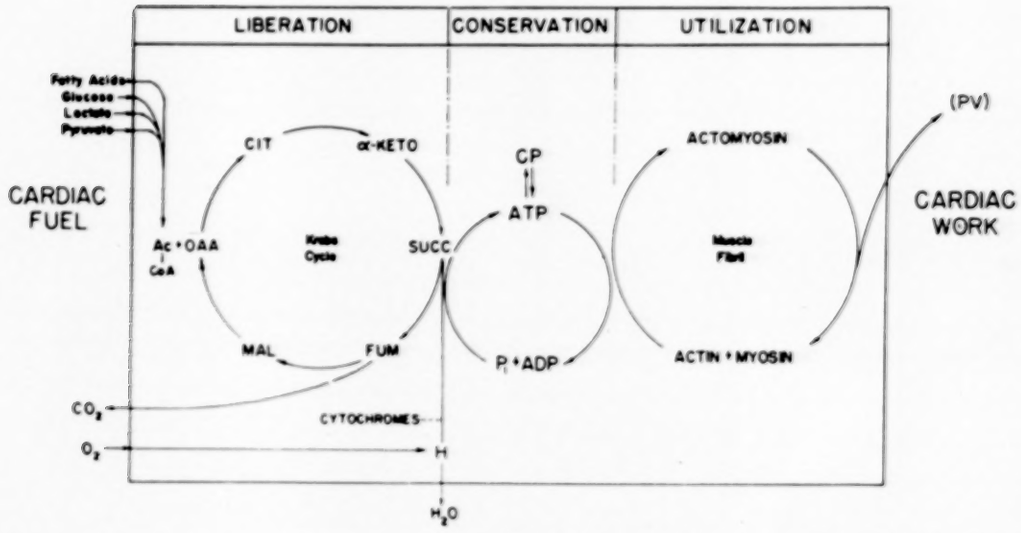

FIG. 1. Schema of energetics in cardiac muscle. (From OLSON, R. E. and PIATNEK, D. A. *Ann. New York Acad. Sc.*, 72: 466, 1959 [*92*].)

chemical events underlying the shortening of the myofibril; third, to present a hypothesis to account for heart failure on the basis of alteration in the physicochemical properties of the contractile proteins; and fourth, to summarize briefly the data available on the function of the cardiac glycosides.

CONTRACTILE PROTEINS

Myosin. Myosin was first extracted from skeletal muscle by Kühne and recognized to be a globulin soluble in salt solution and not in distilled water. It is the major protein of the myofibril. Myosin from striated muscle is soluble in salt solutions at ionic strengths above 0.3 at neutral pH and is completely precipitated at ionic strengths of 0.05. From these properties it is reasoned that at physiologic pH and ionic strength, at least 90 per cent of myosin must be in the gel form.

Edsall [*16*] and Weber [*17*] purified myosin and studied its physicochemical properties. Difficulties have been encountered in determining the size, shape and molecular weight of myosin from skeletal muscle because of its extreme asymmetry, easy denaturation, and proneness to aggregation at room temperature. Estimates of the molecular weight of rabbit skeletal myosin have ranged from 1,500,000 [*18*] to 389,000 [*19*]. Most recent measurements of the sedimentation and diffusion and light scattering of rabbit skeletal myosin carried out at 4°C. yield values of from 400,000 to 500,000 [*20–23*]. Estimations of length and width from light scattering, viscosity and diffusion data give a mean value of about 1,500 Å × 25 Å with an axial ratio of 60. The electrophoretic mobility is 2.8×10^{-5} cm.^{2}/volts/second and the isoelectric point is at approximately 5.4.

The ATPase activity of myosin was discovered by Engelhardt and Ljubimova in 1939 [*24*] and the significance of this discovery in relation to the problem of chemomechanical coupling was recognized immediately. In view of the probable importance of the ATPase activity of myosin in the contractile cycle, this property of myosin has been studied extensively. The enzymatic activity of myosin is a sensitive indicator of the intactness of the molecule and is dependent upon the presence of —SH groups in the protein [*25*]. Skeletal myosin contains approximately 40 —SH groups per mole and approximately one-third of these appear to be freely reactive and not essential for ATPase activity. The —SH groups revealed by guanidine treatment are essential and when these groups are titrated with parachlormercuribenzoate (PCMB) or N-ethylmaleimide, the ATPase activity decreases in parallel with the decrease in —SH groups. In fact, Kielley and Bradley [*26*] have observed that the ATPase activity of myosin in the presence of calcium increases as the most reactive —SH groups are titrated and thereafter inhibition occurs.

Myosin ATPase is also affected by its ionic environment. Calcium ion at 5×10^{-3} M is a potent activator of purified myosin, and in its

presence myosin ATPase has two pH optima, one at pH 6.4 and another at pH 9. Potassium ions activate slightly and sodium ions antagonize the effect of potassium. Mg^{++} ions at concentration of *ca* 5×10^{-3} M greatly inhibit purified myosin ATPase. Paradoxically, Mg^{++} ions activate actomyosin ATPase, formed by combining actin with myosin. Since actin has no ATPase activity, this effect appears to be due to a modifying effect of actin upon the activity and activation potential of the enzyme. Perry [*27*] has shown that the ATPase activity of isolated myofibrils of skeletal muscle is Mg^{++}-activated, and has calculated that the ionic environment of the cell interior (0.012 M Mg^{++} and 0.006 M Ca^{++}) would permit the Mg^{++}-activated but not the Ca^{++}-activated ATPase to function [*28*]. The maximum rate of ATPase activity observed by purified myosin (Ca^{++} activated) is a Qp (μL.P_i liberated per milligram protein per hour) of 15,000 at 37°c., although more usual values range from 4,000 to 8,000 [*29*]. The upper rate calculated from production of P_i from ATP in the intact working skeletal muscle is 10^{-3} M P_i/gm. tissue/minute and for the heart about 10^{-4} M P_i/gm./minute. Skeletal Mg^{++}-activated myofibrillar ATPase can liberate about 4×10^{-4} M P_i/gm. muscle/minute from ATP under *optimal conditions* [*30*]. Similar studies have not been carried out in the heart muscle. The enzymatic specificity of myosin ATPase is such that high specificity for the terminal phosphate of the triphosphate moiety is present, but broad specificity at the nucleotide end exists. Inosinetriphosphate, uridinetriphosphate, cytidinetriphosphate, guanosinetriphosphate and acetyl ATP are all readily hydrolyzed by myosin [*31,32*]. The activation of myosin ATPase by EDTA is thought to be due to chelation with a metal, possibly magnesium, which is tightly bound to myosin.

It has been found by several investigators [*33,34*] that short periods of treatment of rabbit skeletal myosin with proteolytic enzymes (trypsin, chymotrypsin) result in striking changes in the properties of the protein and fission of the myosin molecule into several discrete fragments. There is a decrease in the viscosity of the solution and increase in water solubility of the products. Examination of these myosin solutions in the ultracentrifuge after short (three to twelve minutes) tryptic digestion results in the appearance of two components; one component sedimenting slower and the other slightly faster than the intact myosin molecule [*35*]. The heavy faster component, H-meromyosin (HMM), has been shown to have a molecular weight of approximately 324,000 [*36*]; the lighter one, L-meromyosin (LMM), has been found to have a molecular weight of about 110,000. It is now thought that one L-meromyosin plus one H-meromyosin, plus a small peptide liberated in the digestion add up to one full myosin molecule. It is interesting that the full ATPase activity of myosin and the actin combining power are contained in the heavy HMM. LMM has neither ATPase activity nor actin combining power but is similar to myosin in solubility characteristics, whereas HMM is considerably more soluble in solutions of low ionic strength. Szent-Györgyi et al. [*37*] have recently found that the light meromyosin fraction can be fractionated further with ethanol to yield a fraction called light meromyosin Fraction 1 (LMM-1) which has a molecular weight of 120,000, behaves as a homogeneous material in hydrodynamic studies and on diethylaminoethyl cellulose columns, and has an optical rotation consistent with that of a fully coiled α-helix. All the meromyosins, and myosin itself to a lesser degree, break down to smaller units in the presence of urea, guanidine salts and other reagents that rupture hydrogen bonds. This behavior suggests that linkages other than covalent ones hold a considerable portion of the myosin molecule together.

Kielley and Harrington [*38*] have shown that rabbit skeletal myosin on addition of 5 M guanidine HCl dissociates into completely unfolded α-helical monomers of molecular weight 206,000 and approximately 8×2620 Å in size. These authors suggest that three of these monomers form a triple stranded rope which is partially doubled back on itself to yield a native myosin molecule 1650×22 Å, weighing 619,000. Equally plausible in the light of many estimates that the molecular weight of skeletal myosin is of the order of 440,000 is the view that the native skeletal myosin is a dimer of the primary peptide chain.

Studies of normal dog heart myosin in my laboratory [*39*] have shown that although cardiac myosin has roughly the same solubility characteristics as skeletal myosin, its molecular weight is distinctly lower. Estimates of molecular weight from sedimentation and diffusion constants, and independent estimates from equilibrium sedimentation studies and light scattering

TABLE I

PROPERTIES OF THE MYOSINS FROM NORMAL STRIATED MUSCLE

Protein	$S^\circ_{20,w}$	$D^\circ_{20,w}$	$[\eta]$	Length	Width	Molecular Weight	ATPase Q_p (25°)
Skeletal myosin*	6.2	1.05	1.9	1,500	24	440,000	3,000
Cardiac myosin†	6.1	2.45	0.5	690	28	226,000	400

* From the skeletal muscle of the rabbit. † From the cardiac muscle of the dog.

measurements have been in good agreement, with a value of approximately 225,000 for the molecular weight. The dimensions of the molecule calculated from viscosity and from sedimentation and diffusion measurements were 690 × 28 Å. This particle weight is within experimental error of the monomer of skeletal myosin reported by Kielley and Harrington [*38*] and its dimensions could be duplicated by appropriate folding of this monomer. The amino acid composition of normal cardiac myosin is practically indistinguishable from that of rabbit skeletal myosin [*40–42*]. The higher estimates of molecular weight for cardiac myosin from the heart of a dog [*43,44*] appear to be due to the use of aggregated or impure preparations. The contrasting properties of skeletal and cardiac myosin are shown in Table I. It has also been repeatedly demonstrated that the ATPase activity of cardiac myosin is only one-fifth to one-tenth that of skeletal myosin [*39,45–47*]. The ATPase activity of cardiac myosin responds to given ions, EDTA and actin in a manner qualitatively similar to that of skeletal myosin. Tryptic digestions of cardiac myosin carried out in our laboratory [*48*] show, furthermore, that the products obtained in no way resemble the meromyosins derived from rabbit skeletal myosin. These various lines of evidence clearly indicate that cardiac myosin is related to (possibly as monomer is to dimer) but not identical with skeletal myosin. The differences in ATPase activity of the two proteins suggest other differences in structure, apart from molecular weight.

Actin. Actin was discovered by Banga and Szent-Györgyi in 1942 [*49*]. Actin is obtained with more difficulty than myosin from muscle and requires prolonged extraction with hypertonic salt solutions at a slightly alkaline pH. The viscous actomyosin complex is usually removed under these conditions but actomyosin can be dissociated *in vitro* by ATP to yield actin and myosin. An acetone-dried muscle residue remaining after the partial removal of myosin may also serve as starting material for extraction of actin, which may be extracted with distilled water at neutral or slightly alkaline pH to yield actin in the globular form (G-actin). G-actin can be polymerized to a fibrous form (F-actin) by adding 0.1 M KCl and traces of magnesium chloride. Actin is unique among the fibrous muscle proteins in that it aggregates in the presence of salts and dissociates into monomers in their absence [*50*]. Globular actin has a molecular weight of 70,000 and molecular dimensions of 290 × 24 Å. In the presence of ATP, magnesium ions and low concentrations of KCl, G-actin dimerizes to form an aggregate of molecular weight of 140,000 which is 590 × 24 Å. After removal of the ATP the dimer further polymerizes to a very long fibrous protein of molecular weight of the order of 1.5 to 3 × 10^6 [*51,52*] which lies in the thin secondary filaments of the sarcomere visible in the electron microscope. If ATP bound to G-actin is dephosphorylated by potato apyrase [*13*] the ability of actin to polymerize is lost. G-actin contains considerable amounts of bound ATP. During the conversion of G-actin to F-actin the nature of the bound nucleotide changes as ATP undergoes dephosphorylation to ADP, namely:

$$n\ \text{G-actin} + n\ \text{ATP} \rightleftarrows \text{F (G-actin} - \text{ADP})_n + n\ P_i$$

While actin itself has no ATPase activity, inorganic phosphate is liberated during polymerization. Inactivation of —SH groups of actin with organic mercurials inhibits polymerization.

Cardiac actin appears to be similar to but not identical with skeletal actin [*53–56*]. Cardiac G-actin appears to polymerize to F-actin with much greater difficulty than that from skeletal muscle. It has been reported by Horváth [*57*] that cardiac glycosides potentiate the polymerization of cardiac G-actin in the presence of ATP, an effect not noted with skeletal actin.

The significance of the latter observation in relation to the contractile cycle *in vivo* is not clear since it is assumed that actin is present in the thin filaments as F-actin throughout the contractile cycle.

Actomyosin. When solutions of actin and myosin are brought together a complex of actomyosin is formed. It is characterized by a viscosity higher than that of the sum of the component proteins, a high sedimentation constant, and a very high molecular weight. Light scattering studies indicate an average weight of the order of 20×10^6 [*58*]. The solubility of actomyosin is somewhat less than that of myosin, actomyosin precipitating at a higher KCl concentration than myosin. The combination of actin and myosin is not stoichiometric although the usual combination is about one part of actin to five of myosin, which means that F-actin of a molecular weight of about 2 million combines with 30 myosin molecules to give a macromolecule of 17 to 18 million in molecular weight. This *in vitro* actomyosin is probably a physiologic artefact, however, since recent evidence suggests that myosin and actin are segregated in living muscle into the thick and thin filaments, respectively, of the sarcomere.

Combination with actin is one of the most sensitive properties of myosin. Binding the —SH groups of myosin destroys its capacity for ATPase activity and its capacity for reaction with actin. If the actomyosin gel is treated with ATP in the presence of low amounts of magnesium ion, shortening of the gel associated with splitting of ATP occurs, and the actomyosin complex dissociates into its component proteins [*59*].

Tropomyosin. Tropomyosin, a third myofibrillar protein, was isolated by Bailey in 1948 [*60*]. It appears to be a ubiquitous component of the myofibril and has been prepared from a large variety of both striated and smooth muscle from vertebrate and invertebrate forms. In vertebrate skeletal and smooth muscle it comprises about 10 to 12 per cent of the myofibrillar protein [*61*], but in cardiac muscle from the pig it amounts to about 4.2 per cent [*62*]. Tropomyosin is easily extracted from muscle which has been dried by extraction with ethanol and ether. It is soluble in solvents of moderate ionic strength and is purified by repeated iso-electric precipitation. The tropomyosin monomer appears to be a relatively small protein with molecular weight of 53,000 [*63*]. Its dimensions are 12×400 Å and its physical properties suggest that it is a rigid α-helix. The protein polymerizes in the absence of salts, forming a gel. In the heart of the pig the minimum molecular weight was found to be somewhat higher, i.e., 89,000 [*64*]. Tropomyosin forms dissociable complexes with ribonucleic acid to constitute a nucleotropomyosin. Tropomyosin is the only fibrous protein which forms true crystals (containing approximately 90 per cent water). It has no enzymatic activity and does not form a complex with either actin or myosin. Tropomyosin is distributed largely in the I and A bands of skeletal muscle [*65*] and appears to be associated with actin in the thin filaments *in vivo*.

The comparative studies of Sheng and Tsao [*62*] have shown that, in general, the tropomyosin content of smooth muscle is higher than that of striated muscle in the same species, and it has been suggested by Bailey [*10*] that tropomyosin plays a part in the holding function of such muscles. In the smooth adductor of the oyster, for example, in which tropomyosin comprises from 30 per cent of the total protein, the relaxation phase is very slow in comparison with the speed of contraction. The "catch" muscles of mollusks contain a related protein called paramyosin [*66*], which is extracted with high concentrations of neutral salts and has a solubility behavior similar although not identical to tropomyosin. It appears to be a higher molecular weight protein which is highly asymmetric and which also consists of a single α-helix, with molecular weight 131,000. The physicochemical relationship of paramyosin and tropomyosin suggest that they both play a role in the "holding function." The low content of tropomyosin in cardiac muscle, where "holding" is least desirable, is consistent with this hypothesis.

MECHANISM OF CONTRACTION

The thermodynamic aspects of the various mechanisms of contraction which have been proposed have been considered elsewhere in this symposium [*67*]. The precise molecular interrelationships of the contractile proteins in the mechanisms postulated are not at all clear. A theory of contraction will be satisfactory only to the extent that it accounts for all the structural, biochemical and thermodynamic phenomena observable in living muscle tissue. Most of the hypotheses which have been advanced have been designed to account for the molecular events which occur in striated muscle and are relevant to the events in cardiac muscle. The

hypotheses regarding the basic events in the contractile process can be divided into two groups: (1) those that specify folding in one or more of the contractile proteins, and (2) those that specify sliding of filaments containing these proteins within the sarcomere to achieve a decrease in the length of the sarcomere. None of these can, at this time, be said to be generally accepted. The most plausible hypothesis currently under consideration is one advanced independently by A. F. Huxley and Niedergerke [*68*] and H. E. Huxley and Hanson [*69*], and elaborated in later papers [*70,71*]. This hypothetical mechanism will be presented in detail as a background for discussion of the possible role of the contractile proteins.

Electron microscopic studies of the ultrastructure of the muscle undergoing passive stretch or contraction down to 60 per cent of resting length reveal little if any change in the length of the A-bands. All of the change appears to take place in the I-bands. At maximum contraction the Z-membrane appears to fuse with the edge of the A-band and the central H-band becomes lighter giving the illusion of a reversal in striation. With maximum shortening (30 per cent of resting length) or in contracture the A-band may also shorten appreciably. These observations led to the view that the process of contraction was characterized by the sliding of interdigitating filaments. (Fig. 2.)

As has been pointed out earlier, two types of filaments exist in the myofibril. The thicker ones, 100 Å in diameter, coincide with the A-bands of the sarcomere and have been shown to consist mainly of myosin [*72,73*]. The thin filaments appear to originate in the Z-membrane and extend toward the center of the sarcomere where they do not, at resting length, meet the opposing thin filaments from the opposite Z-membrane. There is some evidence that the thin filaments are connected in the H-band by a tenuous S-filament. The thin filaments appear to be composed mainly of actin [*69–71*] and possibly tropomyosin [*65*]. Chemical analysis of the isolated myofibril of the rabbit [*61*] indicates that the protein composition of the myofibril is as follows: myosin 50 to 55 per cent; actin 20 to 25 per cent; tropomyosin 10 to 15 per cent; and other proteins 5 to 10 per cent. The identity of the protein of the Z-membrane is unknown, although it is estimated to represent about 5 per cent of the myofibrillar protein. In skeletal muscle the myosin filaments are 100 × 15,000

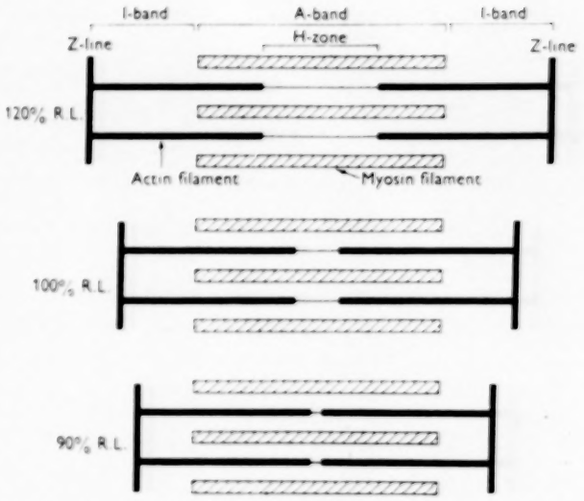

FIG. 2. Diagram showing behavior of actin and myosin filaments during changes in muscle length. R. L. indicates resting length. (From HUXLEY, H. E. *Endeavor*, 15: 177, 1956 [*71*].)

Å, which would accommodate approximately 300 molecules of skeletal myosin 25 × 1500 Å. In cardiac myosin the thick filaments are somewhat shorter but the size of cardiac myosin is also smaller, the filaments being 100 × 10,000 Å and the molecule 28 × 700 Å, indicating that the thick filaments in cardiac muscle contain about 400 molecules of cardiac myosin. The thin filaments appear to be composed of several strands of F-actin and tropomyosin.

According to A. F. Huxley [*70*] the interaction of the filaments in the contractile process is made possible by the presence of reactive sites on the myosin filament which can combine with reactive sites on the actin filament. "Feet" protruding from the thick filaments have been seen in the electron microscope, and could represent entwined myosin peptides protruding from the body of the macromolecular filament. It is postulated that the linkages between the sliding members are formed spontaneously but are broken only by the input of energy from metabolic sources. This is not difficult to imagine if it is supposed that the reactions are catalyzed by enzymatic activity intrinsic to one of the contractile proteins, as ATPase is to myosin. The details of the sliding model have been reviewed elsewhere in this symposium [*67*].

The biochemical events which may be postulated to drive the sliding model are as follows:

$$\text{Actin} + \text{Myosin} \rightarrow \text{Actomyosin} \qquad (1)$$

The myosin is thus regarded as "energy rich" and capable of "pulling" the actin filament

towards it by combining with it in the highly oriented manner imposed by the ultrastructure of the myofibril. The actomyosin bond thus formed is broken (either in more vigorous shortening or by relaxation) by reaction of the actomyosin with ATP as follows:

$$\text{Actomyosin} + \text{ATP} \rightarrow \text{Myosin-ATP} + \text{Actin} \quad (2)$$

The myosin-ATP complex, which may be regarded as an enzyme-substrate complex, is then split by the ATPase activity of myosin as follows:

$$\text{Myosin-ATP} + H_2O \rightleftarrows \text{Myosin} + P_i \quad (3)$$

Levy and Koshland [*74*] have studied reaction (*3*) with H_2O^{18} and have shown that the kinetics are consistent with the formation of an intermediate short-lived phosphomyosin which may be the key to the transfer of energy of ATP to the contractile protein. These workers found that this exchange reaction occurred in the presence of magnesium but not with actin alone or with myosin in the presence of calcium. Since magnesium is essential for the myofibrillar ATPase system, it is likely that phosphomyosin is formed in the intact muscle cell. The question of the direction of the movement of the filaments must depend upon the state of activation of the Z-membrane which distributes the action potential of the membrane to the contractile elements by way of the endoplasmic reticulum [*75*]. It is also of interest that this model obviates the necessity of designating the time of the contractile cycle when the splitting of ATP occurs; it occurs during all movements of the filaments in order to permit the making and breaking of the points of attachment between actin and myosin.

A considerable amount of attention has been given in recent years to the identification of relaxing factors in striated muscle [*7*]. Marsh [*76*] noted that if skeletal muscle was homogenized and the original supernatant replaced with fresh 0.16 M KCl, an increase in ATPase activity and renewed responsiveness to ATP was observed. He concluded from this that a factor present in muscle promotes relaxation by inhibiting actomyosin ATPase. It appears that factors in muscle which promote relaxation increase ATP and decrease actomyosin ATPase, resulting possibly in an enzymatically inactive ATP-protein complex. Of the known relaxing factors (none of which is as yet conclusively identified with the physiologically active one), EDTA binds magnesium and hence inactivates myofibrillar ATPase [*77*]; myokinase and creatine [*78*] form ATP, inorganic pyrophosphate inhibits actomyosin ATPase, and the microsomal fraction of Mueller [*79*] causes marked inhibition of actomyosin ATPase. Since these effects are very sensitive to the presence or absence of Ca^{++} and Mg^{++}, it is possible that ATPase inhibition by relaxing factor is present in resting muscle and that ion shifts which induce activation of the membrane and reticulum reverse the effect and permit contraction to occur.

The sliding model provides a new and stimulating hypothesis for contemplation. It is certainly not established but at the moment have so many attractive features that it will be studied intensively in the future. The biochemical events which are postulated to occur also are not firmly established but provide a working hypothesis for future study. The other models which have been proposed to account for chemomechanical coupling in the myofibril has strengths and weaknesses which deserve mention. Szent-Györgyi [*80*] visualized a folding model in which actin and myosin combine in the presence of ATP and shorten, much as actomyosin gels or glycerinated fibrils do *in vitro*. It is unlikely, however, from the electron microscopic studies that actin and myosin combine in the myofibril as they do *in vitro*. The actomyosin complex as it is studied in solution may be a physiologic artefact. More recently Szent-Györgyi [*15*] has suggested that muscular contraction may be a submolecular phenomenon involving protomyosin (fragments of myosin) and the triplet state of their electrons. The triplet state occurs when an electron is raised to an excited state by the absorption of energy which reverses its spin. The excited electron is trapped because it cannot drop back to the original energy level of its partner which is spinning in the same direction and as a result the lifetime of the excited state is lengthened about a million-fold. The molecule which contains the uncoupled electrons is in an unbalanced and more reactive state, like a free radical. In Szent-Györgyi's view, molecular contraction would be a quantum mechanical process involving displacement of myosin fragments [*15*].

Morales and Botts [*81*] have suggested that the primary event in muscular contraction is a folding of a Mg-myosin complex to which ATP is adsorbed (acting as a polyelectrolyte) in an electrostatic environment altered by passage of the action potential. The hydrolysis of ATP is visualized as occurring at the end of contraction

to permit rebuilding of the appropriate polyelectrolyte structure for a relaxed myosin. This view, postulating deformation of myosin, is contradicted most directly by the observation that the A-bands of muscle, which contain myosin, do not appreciably shorten under most physiologic conditions.

Straub [*13*] has suggested that polymerization of G-actin to F-actin is a crucial reaction in muscular contraction. This idea, not popular in the last decade, may deserve re-examination in the light of the Huxley model with its assignment of a more important role to the actin filaments. The early hypothesis of Astbury [*82*] that contraction results from a phase change in myosin has not been confirmed experimentally. Bailey [*41*] has suggested that the function of tropomyosin may be to control the rate of operation of the contractile cycle by interacting with actin.

With regard to energy sources for contraction, it seems indisputable that ATP is the ultimate source. Claims that other labile phosphate compounds may be interposed between ATP and the contractile cycle have not been fully substantiated [*83,84*]. The efficiency of the conversion of ATP to mechanical work in cardiac muscle varies widely, from about 10 to 60 per cent, indicating that the "coupling" of ATP utilization to actomyosin function is considerably looser than the coupling of hydrogen transport to ATP formation [*85*]. Under normal conditions the over-all efficiency of the heart varies from 10 to 30 per cent, although in failure it may fall to as low as 3 to 5 per cent.

CONGESTIVE HEART FAILURE

A variety of disorders exist in man and in experimental animals in which the contractility of muscle is impaired. None is more dramatic than congestive heart failure. Evidence is mounting that most of the common types of cardiac failure are due to defects in energy utilization [*86,87*], i.e., failure of the myofibril to assimilate phosphate bond energy or to shorten properly in the contractile cycle. A decade ago, it was suggested by Olson and Schwartz [*87*] that cardiac failure could be divided biochemically as well as hemodynamically into two forms: (1) high output failure or metabolic heart failure; and (2) low output failure or nonmetabolic heart failure. The high output syndrome occurs in metabolic disorders such as anemia, thyrotoxicosis, beriberi, uremia and cholemia, and it features a general biochemical defect which may be identified in the schema of reactions leading to ATP formation. In most common types of low output failure the disease is restricted to the heart itself and does not involve the reactions leading to ATP formation. In low output failure secondary to valvular disease, previously normal cardiac muscle has been obliged to work at a mechanical disadvantage for a period of time. The myocardium undergoes dilatation and hypertrophy, and ultimately is unable to perform its work. The time required for induction of failure is generally related inversely to the seriousness of the mechanical disadvantage at which the heart labors. Cardiac failure of this type may result from congenital anomalies of the valves and great vessels, valvular disease or hypertension. It may also occur at "normal loads" in atrophic or infarcted cardiac muscle. In these conditions the energy production of the heart is normal. The glycolytic enzymes of the cytoplasm and the dehydrogenases and electron transport chain of the sarcosome appear to function effectively. That normal energy production can occur in association with cardiac failure has been shown in the heart-lung preparation [*88–90*], the open chest of the dog [*91*], the intact dog [*92*], and in the human subject with hypertension or valvular disease [*93–95*]. Using cardiac catheterization Blain, Goodale et al. [*94,95*] found no decrease in coronary blood flow, oxygen extraction or substrate utilization in patients with congestive heart failure and roentgenographic evidence of left ventricular enlargement. The inability of these investigators to show any defect in myocardial oxygen consumption and failure does not support the older view of many pathologists, summarized by Harrison [*96*], that anoxia within the hypertrophied cardiac muscle fiber is responsible for the failure of contractility.

Olson et al. [*1,92,97*] have studied the metabolic behavior of the failing heat in dogs subjected to cardiac valvular surgery. Chronic low output congestive heart failure characterized by edema, ascites, weakness, reduced tolerance to exercise, cardio- and hepatomegaly, with ultimate elevation of end diastolic filling pressures in both the right and left ventricles (Fig. 3), was produced in dogs by avulsion of the tricuspid valve and stenosis of the pulmonary artery. Although right-sided heart failure is initially induced in this way, failure eventually becomes generalized, as indicated by depressed ventricular

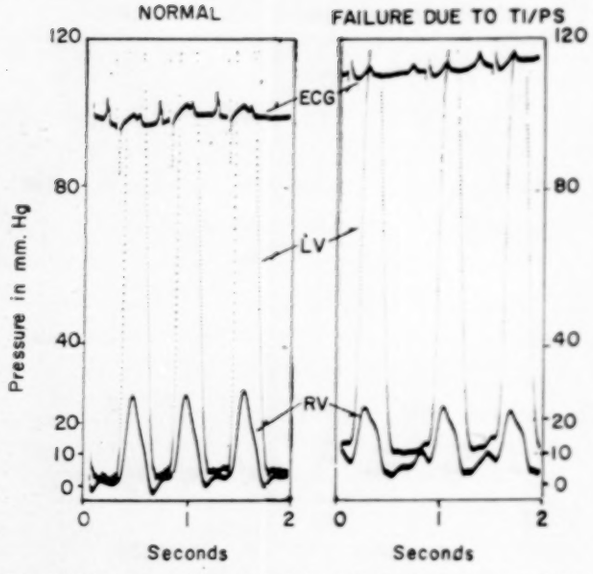

FIG. 3. Tracings of left and right ventricular pressures in a normal dog and in a dog with congestive heart failure due to tricuspid insufficiency and pulmonic stenosis. The panel on the left presents the normal tracings; the panel on the right shows the elevated end diastolic filling pressures in both the right and left ventricle. Electrocardiographic tracing is included in each case. (From OLSON, R.E. et al. *Circulation*, in press [*104*].)

function curves in both ventricles [*98*] and a positive inotropic response to digitalis in both chambers. More recently, primary left heart failure due to aortic insufficiency and mitral insufficiency has also been produced [*104*].

The cardiac work and metabolism of a series of dogs in congestive heart failure due to valvular disease was compared with a series of healthy control animals. The cardiac output was found to be decreased in the animals with congestive heart failure associated with valvular disease but coronary blood flow and myocardial oxygen usage were unchanged from normal. The extraction of glucose, lactate and pyruvate was slightly increased in the dogs with congestive heart failure, so the total contribution of carbohydrate to energy production was increased and the contribution of fatty acids was slightly reduced [*92*]. A study of the distribution of high energy phosphate compounds in ventricular muscle from normal animals and those in failure showed no difference in the pattern of distribution in the normal and failing myocardium. The fact that ventricular ATP and CP levels do not change in failure due to valvular disease, in agreement with the findings in the failing heart-lung preparation [*99*], suggests that availability of high energy bonds from myocardial contraction is not limiting in this syndrome. Brody, Palmer and Bennett [*100*] compared the ability of ventricular muscle from failing and non-failing heart-lung preparations to carry on oxidative phosphorylation from pyruvate *in vitro* and found no difference in the P:O ratios. We have confirmed this finding in a study of mitochondria from normal and failing dog heart ventricle in our laboratory.

In view of the inability of several laboratories to find evidence for a defect in energy production or conservation in the failing heart in either man or in the experimental animal, a study of the contractile proteins in experimental heart failure was undertaken by us some years ago [*97*]. Myosin was isolated from ventricular muscle from normal animals and from those with congestive heart failure by the procedure of Szent-Györgyi [*14*]; it was rigorously purified by repeated dilutions and ultracentrifugation to remove any residual actomyosin; and then it was subjected to numerous physicochemical measurements including sedimentation (velocity and equilib-

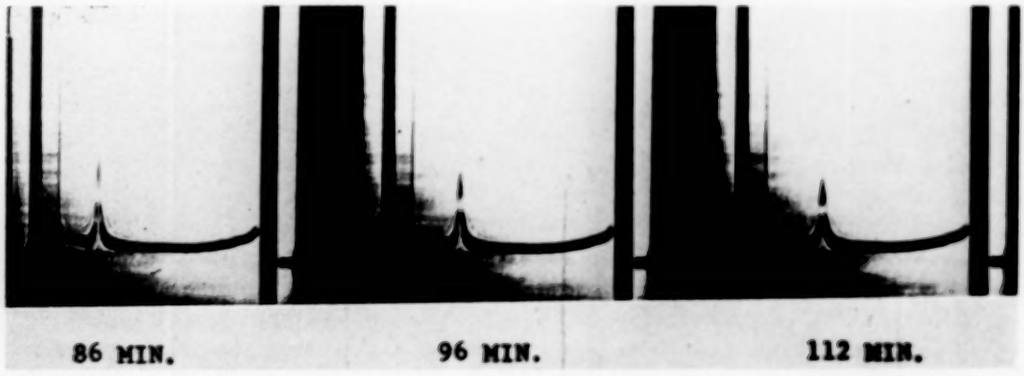

FIG. 4. Homogeneous cardiac myosin from a normal dog as separated by ultracentrifugation. Conditions were as follows: protein concentration 0.440 per cent; rotor speed 56,100 revolutions per minute; ionic strength 0.6; pH 6.8; $S_{20,w}$ = 4.77. (From OLSON, R. E. *J. Chron Dis.*, 9: 442, 1959 [*1*].)

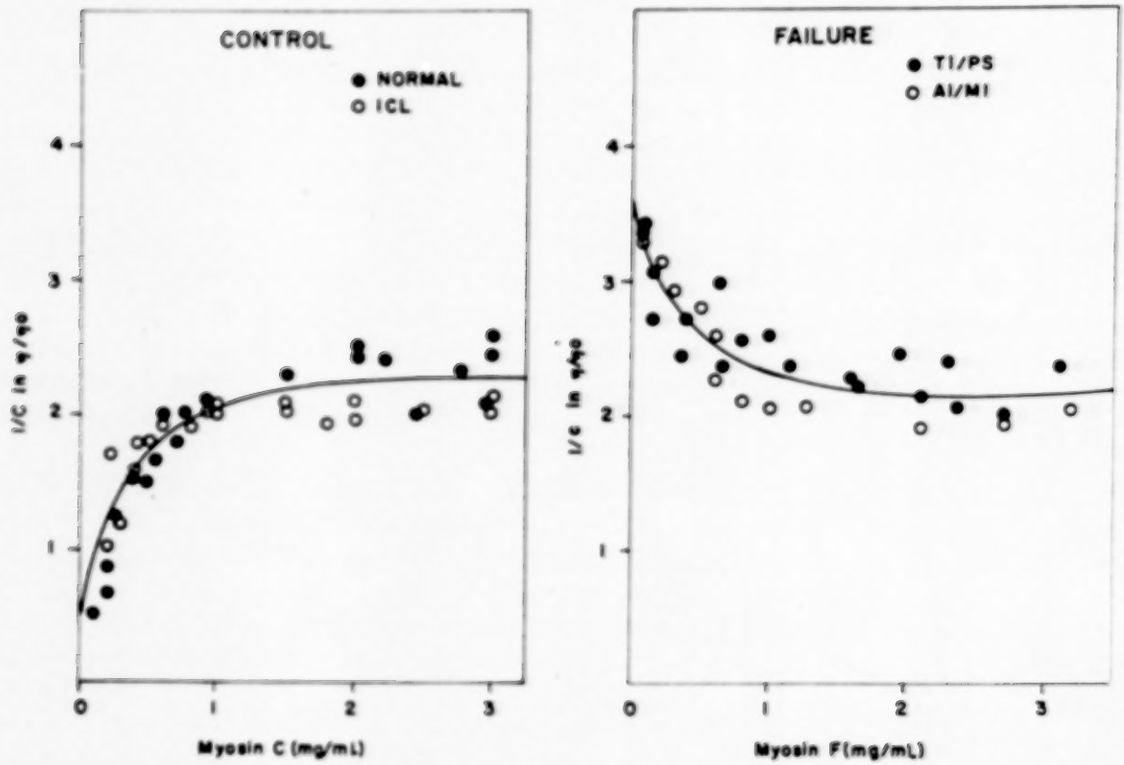

FIG. 5. Viscosity measurements in dog heart myosin as a function of concentration. Values in the left panel were obtained from preparations of normal animals (filled circles) and animals with ascites due to inferior vena cava ligation (open circles). Values in the right panel were obtained from preparations of animals in congestive heart failure due to tricuspid insufficiency and pulmonic stenosis (filled circles) and aortic insufficiency and mitral insufficiency (open circles). Conditions were as follows: temperature 1°c.; ionic strength 0.6 M KCl; pH 6.8. (From OLSON, R. E. et al. *Circulation*, in press [*101*].)

rium), diffusion (free and boundary-spreading in the ultracentrifuge), intrinsic viscosity, light scattering behavior and ATPase activity. All of the myosin preparations from normal animals appeared homogeneous in the ultracentrifuge (Fig. 4) and by electrophoresis. Marked differences in the physicochemical behavior of myosin preparations from the normal and failing heart were noted. The physicochemical data for normal cardiac myosin (myosin C) and for the myosin isolated from the failing heart (myosin F) are summarized on Table II. These constants, together with the light scattering behavior, led to estimation of a molecular weight of 226,000 for myosin C and 695,000 for myosin F [*101*]. The ATPase activity per gram of myosin was unchanged. The changes in viscosity as a function of concentration are quite different for myosin C and myosin F. (Fig. 5.) Normal cardiac myosin appears to disaggregate in very dilute solution and extrapolates to an intrinsic viscosity of 0.5 whereas myosin isolated from the failing heart shows a reciprocal behavior and appears to undergo aggregation in dilute solution, reaching a very high intrinsic viscosity of 3.63. This behavior has also been noted by Davis et al. [*44*]. Studies of amino acid composition of myosin C and myosin F indicate that the molar ratios of

TABLE II

PROPERTIES OF MYOSIN FROM THE NORMAL AND FAILING DOG HEART

Protein	$S^\circ_{20,w}$	$D^\circ_{20,w}$	$[\eta]$	Length	Width	Molecular Weight	ATPase Q_p (25°)
Myosin C	6.2	2.45	0.5	690	28	226,000	382
Myosin F	6.5	0.82	3.6	2,224	28	690,000	424

the amino acids are very similar in the two proteins. The most consistent change has been an increase in amide nitrogen in the myosin isolated from failing heart, suggesting that the polarity of this protein has been reduced somewhat in association with its increase in size. The change may be written as a polymerization of myosin according to the following equation:

$$3\ \text{Myosin C} + n\ NH_4^+ \rightarrow \text{Myosin F} + n\ H_2O + n\ H^+$$

The stimulus for this change appears to be chronic stretch, to which heart muscle working at a mechanical disadvantage is subjected. The extensive hydrogen bonding of myosin may well be disrupted under such conditions to permit aggregation to occur [*102*].

These data are consistent with the view that an abnormal stable aggregate of myosin C is formed in association with cardiac failure and that this myosin F prevents the formation of an actomyosin with normal contractile properties [*103*]. Working with a much narrower range of physicochemical methods and with admittedly impure preparations of myosin from the normal dog and from the dog with congestive heart failure due to tricuspid insufficiency and pulmonic stenosis, Davis et al. [*44*] drew the conclusions that there were negligible changes in the molecular weight of cardiac myosin from the failing heart. They reported that both normal cardiac myosin and myosin from the failing heart had molecular weights of the order of 5×10^5, a result not in agreement with ours. A critique of their data and conclusions has been published elsewhere [*104*].

Benson [*105*] found that the actomyosin content of the ventricles in mammals in congestive heart failure due to tricuspid insufficiency and pulmonic stenosis was reduced below normal. He noted further that this actomyosin had certain abnormal properties, namely, a decreased change in viscosity per unit of actomyosin upon the addition of ATP, which suggested a lower content of actin. More recently Benson and co-workers [*106*] have found that the *in vitro* contractility of glycerol-extracted muscle strips from both the right and left ventricles of dogs with heart failure due to tricuspid insufficiency and pulmonic stenosis was markedly reduced; a depressed ventricular function curve similar to that observed *in vivo* could be constructed from the data. Since glycerol-extracted muscle contains little else than the basic contractile system and responds to ATP like isolated actomyosin, it seems reasonable to assume that the defect in contractility observed by Benson and co-workers must be due to an alteration in the contractile proteins. Kako and Bing [*107*] noted a similar decrease in the contractility of actomyosin bands prepared from failing human heart muscle postmortem. It seems reasonable to conclude that the biochemical lesion in congestive heart failure of the low output type is an acquired molecular disorder which affects the contractile mechanism.

Polymerization of myosin (both skeletal and cardiac) has been noted to occur *in vitro* [*108*] during denaturation. The extent to which the polymerization observed with cardiac myosin in association with congestive heart failure in the dog is etiologic has not been determined. Myosin F is not obtained from dogs with edema due to inferior vena cava ligation, valvular disease without failure, cardiac hypertrophy without failure, or after extensive treatment with desoxycorticosterone acetate [*104*]. Studies of human myocardia are greatly needed. The extent to which the dog with congestive heart failure due to surgical heart disease is a model for the human subject in low output failure is, for the present, a matter for speculation.

FUNCTION OF THE DIGITALIS GLYCOSIDES

Cardiac glycosides are of great value in the treatment of low output failure, in which the biochemical defect in cardiac muscle appears to reside in the contractile mechanism. The precise mode of action of digitalis, however, is still unknown [*109,110*].

An increase in cardiac efficiency appears to be the most conspicuous effect of the action of digitalis in cases of cardiac failure due to hypertension or valvular disease. Stewart and associates [*111*] demonstrated a rise in cardiac output and work per beat as a result of the administration of digitalis in patients with congestive failure due to multivalvular disease. More recently, Bing and co-workers [*112*] have reported that digitalization of patients with this type of low output failure results in increased outputs without changes in coronary flow or cardiac oxygen consumption. In control patients, strophanthidin decreased the cardiac output.

Although the nature of the basic complex between cardiac glycoside and heart muscle is not known, actomyosin may be involved in this union. Mallov and Robb [*113*] and Bowen [*114*] found that actomyosin solutions exposed to

cardiac glycoside showed better spiraling and shortening than a standard preparation not treated with glycoside. Stutz et al. [*115*] and Edman [*116*], however, found that lanatoside C did not influence the force or speed of contraction of normal glycerol-extracted heart muscle. Horváth and colleagues [*57*] have noted an effect of digitalis therapy upon the actin G → actin F conversion, a reaction which appears to be of relatively minor importance in the contractile cycle. Kako and Bing [*107*] found that Ca^{++} and digoxin, but not digoxin alone, improved the contractility of actomyosin bands isolated from failing hearts at necropsy.

Hajdu and Szent-Györgyi [*117*] have suggested that digitalis glycosides exert their inotropic effects by influencing the permeability of the muscle membrane and thus changing the ionic atmosphere in which actomyosin contracts. During rapid digitalization of normal dogs with acetyl strophanthidin, Regan and associates [*118*] noted marked efflux of potassium in the coronary sinus and a negative inotropic effect. In human subjects with low output congestive heart failure these same investigators [*119*] found a similar efflux of potassium after treatment with acetyl strophanthidin which exerted a positive inotropic effect in these failing hearts.

Conflicting reports of an effect of digitalis therapy on the carbohydrate metabolism of the heart have appeared [*120*]. Wollenberger [*121*] found a decreased extraction of glucose by dog heart slices associated with an increased oxidation rate of glucose to CO_2. Kien and Sherrod [*122*] have reported that digoxin (given in one intravenous dose) appears to hasten the myocardial metabolism of a single dose of 20μc. of glucose-U-C^{14} to hexose phosphates and CO_2. These effects may be related to an action of the cardiac glycosides upon the cell membrane or the mitochondrial membrane to permit flux of substrate or metabolite (glucose, potassium, or cytoplasmic DPNH). It seems far from proved, however, that these effects of digitalis are responsible for the positive inotropic effect in the failing heart.

Blain [*123,124*] found that lanatoside C had no effect upon substrate extraction or oxygen consumption in human subjects with or without congestive heart failure. Wollenberger [*125*] noted no effect of ouabain and digoxin (at therapeutic dosage) upon the ATP and CP stores of cardiac ventricle in the heart-lung preparation and we have made similar observations in intact dogs [*126*]. Rothlin and colleagues [*127*] noted that lanatoside C was positively inotropic in the heart-lung preparation of the dog as long as high-energy phosphate compounds were available. When uncoupling of oxidative phosphorylation was induced by dinitrophenol, the effects of the cardiac glycoside were abolished. Rebar, Rebar and Omachi [*128*] found that daily injections of non-toxic doses of digitoxin for four days in dogs caused a decline in cardiac creatine phosphate levels and suggested that myocardial ATPase activity had been increased by the cardiac glycoside. The observation of Grisolia [*129*] that digitoxin *in vitro* has no effect upon oxidative phosphorylation of rabbit heart sarcosomes supports this view.

In our own studies of high energy phosphates in the myocardium of chronically digitalized normal dogs and dogs with experimental heart failure, a consistent pattern of decrease in CP and ATP has not been noted. Friedman and St. George [*130*] observed that after administration of 1 μg. of digitoxin per gm. body weight to rats, the particulates of heart muscle (mitochondria, microsomes and nuclei) contained negligible quantities of the drug, whereas the cytoplasmic fraction (which contains the contractile proteins) had most of the glycoside present in the heart. We have established that digitoxin administered to dogs in daily doses of about 2.5 mg. is bound to cardiac myosin (in amounts of 4 to 10 μg. per gm.) with sufficient stability to resist elution during purification and dialysis of the preparation [*126*]. In fact, digitoxin-H^3 has been observed to be bound to myosin in a molar ratio of 1 in prolonged incubations *in vitro*. Digitoxin bound to myosin *in vivo* appears to increase the instability of myosin from both normal and failing heart as evidenced by easier denaturation.

The digitalis glycosides undoubtedly have multiple activities in the body depending upon the protein or enzyme to which they are bound and the organ in which the effect is noted. The problem which remains is to determine with more certainty the specific activity of these compounds which accounts for the positive inotropic effect in the failing myocardium.

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The Mechanism of Muscular Contraction*

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MUSCLE fibers convert chemical energy into mechanical energy. Upon activation, a chemical reaction takes place which changes the stable configuration of the structure to one of shorter length. (Fig. 1a.) When the fiber is prevented from moving by an external constraint, the change in configuration is reflected as a contractile force. (Fig. 1b.) If the contractile force exceeds the external load, the fiber shortens, moving the load. In this way energy liberated by the chemical reaction ($M \rightarrow M'$) is converted to mechanical work.

The Force-Velocity Relation. Although an activated muscle fiber resembles a stretched spring or rubber band, it differs from such systems in a very fundamental way: at a given length, the force exerted by a spring or a rubber band does not depend on how fast it is moving; the force developed by muscle at a given length is smaller the faster the muscle is moving.

The dependence of contractile force on shortening velocity is the most characteristic property of muscle. It is familiar experience: we can lift a light object more rapidly than a heavy one, we can run up stairs more quickly without a load than with one.

The experimental arrangement for measuring the relation between force and speed of shortening is diagrammed in Figure 2. The muscle is the sartorius muscle of the frog, the favorite preparation of muscle physiologists; frogs are readily available all over the world and their muscles seem to work the same way as in man [*1*]. The pelvic end of the muscle is held fixed and the tibial end is loaded with a weight. When the muscle is activated by electrical stimulation, it generates force. When the force equals that of the load, P, the muscle begins to shorten at a *constant* velocity, V. Figure 3 shows the relation between force and velocity when the experiment is repeated with different loads. The force is maximum, P_o, when the load is so great that the muscle can not shorten at all ($V = 0$). When the load is less than P_o, the muscle shortens at a characteristic rate which is greater, the smaller the load. When it moves at the maximum steady rate, V_{max}, no force develops at all. Thus the force developed by the contractile mechanism depends on its own state of motion. This is the *force-velocity* relation [*2*].

The Viscoelastic Model. The fall in force with contraction rate gave rise to a viscoelastic model for the contractile mechanism [*3*]. It was assumed that protein filaments within the muscle are immersed in a viscous medium, and that upon activation, these filaments are converted to elastic structures. (Fig. 4.)

Suppose that upon shortening the filaments transfer elastic energy either to the load (as work) or to the internal viscosity (as heat). Now, let Ex be the energy released when the muscle shortens a distance x. The energy transferred to the load P is Px. The energy dissipated as heat will depend on the magnitude of the frictional force which, in turn, depends on the contraction velocity: it will be small for slow motion and greater for rapid motion. If η is the coefficient of viscosity, the frictional force will be ηV and the energy dissipated as heat should be ηVx. The energy balance is

$$Ex = Px + \eta Vx$$

or

$$P = E - \eta V$$

Thus the model provides a (qualitative) explanation for the fall in force with increasing velocity. However, when the key assumption (that E is independent of V) was examined critically, it turned out to be untrue. Nevertheless, the theory was successful in another way: by generating controversy it stimulated a number of elegant experiments which provided valuable clues about the nature of the contractile mechanism.

The Fenn Effect. Consider the experimental arrangement in Figure 2. By measuring the heat as well as the work liberated when the muscle lifts (decreasing) weights to a constant height,

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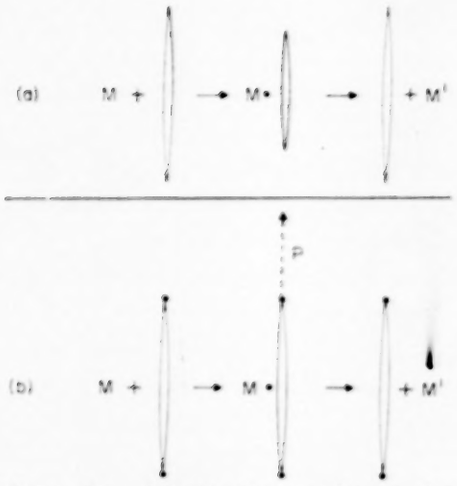

FIG. 1. Conversion of chemical energy into mechanical energy. Chemical energy is liberated in the reaction $M \rightarrow M'$. Shortening is produced in (a); force P, is produced in (b).

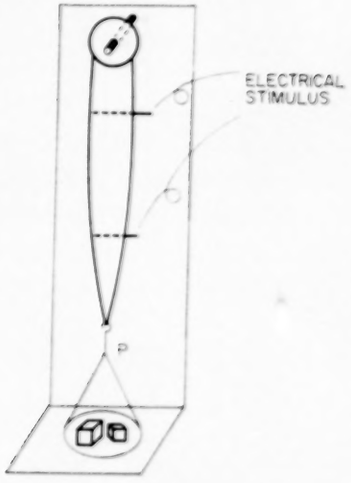

FIG. 2. Experimental arrangement for measuring the force-velocity relation (diagrammatic).

Fenn [*4*] found that the total energy *decreased* as the contraction speed *increased*. This suggested that activation of the contractile mechanism does not endow it with a fixed amount of elastic energy (as assumed in the viscoelastic model); rather, activation makes chemical energy available to the contractile mechanism and the utilization of this energy is somehow affected by the motion itself.

A corollary of the viscoelastic theory, that the heat of shortening increases with velocity, was shown to be untrue in a classic experiment by Hill [*2*]. In Figure 5 the heat output of tetanized sartorius muscle of the frog is shown as a function of time. In curve E the length of the muscle is maintained constant. Heat is liberated at a constant rate: this is the heat associated with activation of the muscle. Curves F, G, H and J show the heart liberated when the muscle is released, at the time indicated by the first arrow, and allowed to shorten a given distance at different rates. Since, at the end of shortening, curves F, G, H and J are all displaced from the isometric case by a constant amount, the extra heat associated with shortening depends only on the distance shortened and *not* on the velocity of

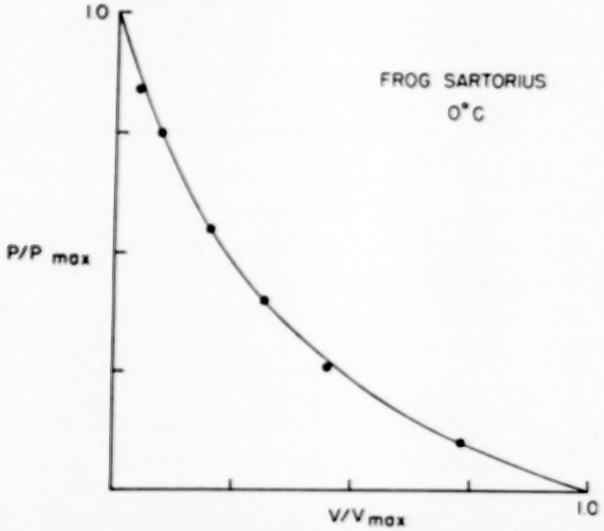

FIG. 3. Relation between force, P, and velocity, V, in living muscle. *Experimental points* measured as shown in Figure 2; *smooth curve* is the force-velocity relation of Hill [*2*].

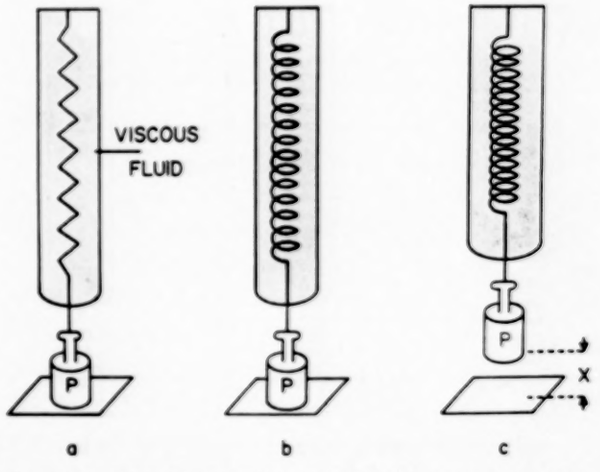

FIG. 4. The viscoelastic model; (a) resting muscle, (b) activation, and (c) shortening.

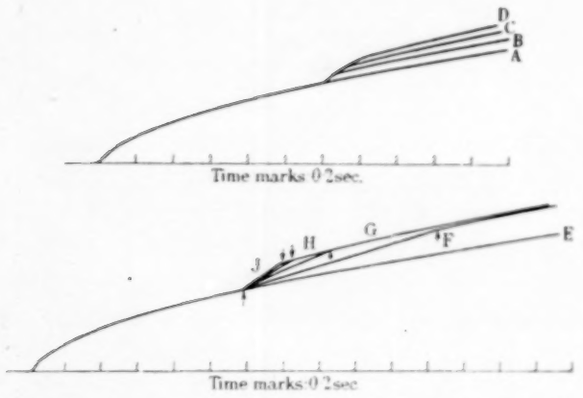

FIG. 5. Heat production in living muscle. Tetanically stimulated sartorius muscle of the frog, 0°C. *Top*, A: isometric contraction. B, C, and D: 1.2 seconds after start of stimulus the muscle is released and allowed to shorten various distances (B < C < D) under *constant load*. *Bottom*, E: isometric contraction. F, G, H and J: the muscle is allowed to shorten the *same distance* under various loads (F > G > H > J). (From HILL, A. V. *Proc. Roy. Soc. London, s.B.*, 126: 136, 1938 [*2*].)

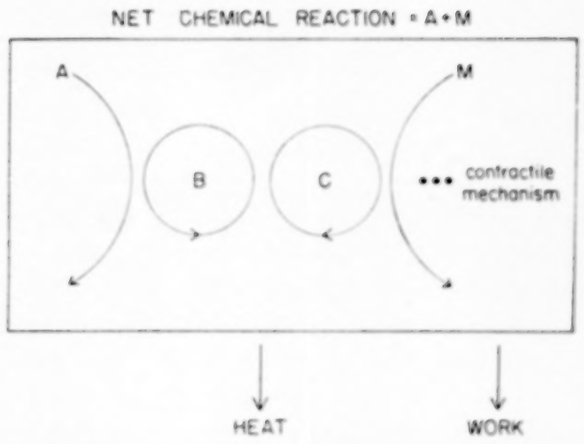

FIG. 6. Scheme for chemical processes associated with muscular contraction.

contraction. Curves B, C and D, the heat output for increasing amounts of shortening, show that the heat of shortening is proportional to the amount of shortening.

In summary, the heat output in activated muscle is

$$Q = mt + ax$$

where mt is the *maintenance heat* and ax is the heat associated with motion of the contractile mechanism, the *shortening heat*. Since shortening heat does not increase with speed, the viscoelastic theory is clearly untenable.

The Mechanochemical Hypothesis. The viscoelastic model focused attention on the internal energy of the contractile mechanism. An alternative view, and one that seems to be closer to the truth, is that the internal energy of the contractile mechanism *per se* is substantially unchanged during steady shortening. In this case the energy flux accompanying motion can be directly related to the chemical reaction driving the process [*5*]. The contractile mechanism is simply a transducer for converting chemical energy into mechanical energy.

This idea is schematized in Figure 6. A sequence of chemical reactions drives the contractile mechanism. One of these reactions, *M*, is closely linked to the contractile mechanism. Since the chemical reactions themselves are linked like a train of gears, the extent of reaction is limited by the turnover of M, which, in turn, depends on the mechanical motion. The total energy produced during contraction depends on the extent of the reaction $A + M$. This chemical energy is partitioned into work and heat. Conversely, the *rate* of chemical reaction can be inferred from the *rate* of energy production.

The purpose of a contraction model is to relate the mechanical motion to the chemical process so that both energy production and its partition into work and heat depend on motion in just the right way. We shall next show what the "right way" is, and then describe how several models manage to do this.

Energy Production in Living Muscle. The fundamental experiments described can be restated to show how the flux of energy, and therefore the chemical reaction, is controlled by the motion of the muscle.

Consider the heat first. (Fig. 5.) Since the total heat of shortening is independent of contraction speed, the *rate* of heat production due to shortening must increase linearly with speed. (Fig. 7, open region.)

The rate of work production ($\dot{W} = PV$) also depends on speed. When the muscle does not move ($V = 0$) it produces no work. Also, when it is unloaded ($P = 0$) no work is done. This ties down the ends of the work flux curve.

Intermediate points can be calculated from the force-velocity relation: $\dot{W}$ is the product of the ordinate (P) and the corresponding abscissa (V) of Figure 3. The result is the shaded region of Figure 7.

Adding the work to the heat, we see that the total energy flux associated with shortening increases monotonically with speed. According

to the mechanochemical hypothesis, the rate of the rate limiting chemical reaction, the one linked with the contractile mechanism, must also increase with speed in exactly the same way.

Summary: The rate of the chemical reaction driving the contractile mechanism increases with V according to Figure 7. At the same time, the force generated by the contractile mechanism decreases with V according to Figure 3.

Structural Basis of Contraction Theories. What mechanism regulates muscle chemistry and force according to speed? How does the contractile mechanism "sense" speed? To answer these questions we turn to the structure of muscle and its change upon contraction.

Electron micrographs of striated muscle reveal the existence of two sets of interdigitating filaments parallel to the fiber axis, one set thicker than the other [6,7]. (Fig. 8.) The muscle striations, or bands, arise from the characteristic arrangement of the thick and thin filaments. The dark A *band* is formed by the array of thick filaments. The light I *band* is formed by the thin filaments. In the middle of the I band the thin filaments seem to be bisected by a narrow region, the *Z line*. At normal muscle lengths the thin filaments extend into the A band, interdigitating there with the thick filaments. The part of the A band not occupied by the thin filaments (or where the thin filaments are even thinner) is the H *zone*. The length of the thick filament is 1.5 μ.; the thin filament is 2 μ. Since the sarcomere at normal muscle length is about 2.5 μ, the I band is normally 1 μ and the H zone is 0.5 μ.

When *passive* muscle is stretched, the thin filaments are withdrawn from the A band: the I band and H zone become greater ($\Delta I = \Delta H$), the A band remains constant. Thus in passive stretch the length of both the thick and thin filaments seems to remain the same, but the extent of interdigitation decreases.

In the inverse experiment, *active* shortening, it is more difficult to decide what happens to the lengths of the myofilaments because the experiment must be carried out in living rather than fixed preparations. When living fibers are stimulated and shortening is monitored with a light microscope, the decrease in length takes place almost entirely in the light I bands, the width of the dark A bands remaining constant [8]. (Fig. 9.) There are two obvious ways for this to come about: the filaments could *slide* along each other (the reverse of passive stretch) or,

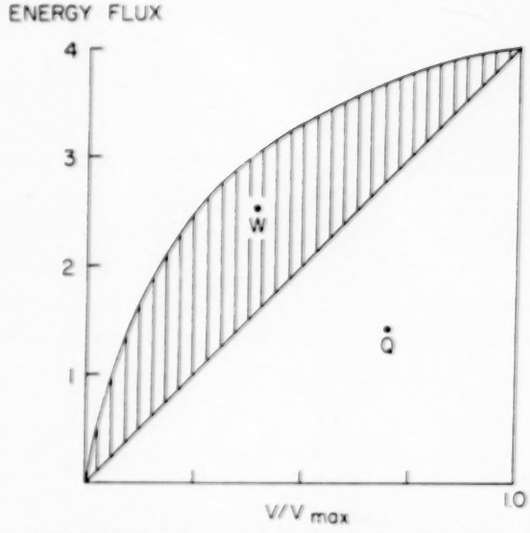

FIG. 7. Relation between energy flux and velocity in living muscle. *Open region:* rate of heat production. *Shaded region:* rate of work production. The unit for the ordinate is the rate of heat production during isometric contraction (the maintenance heat).

after anchoring its ends, the thin filament could shorten by *folding*.* (Fig. 10.) In both schemes only the I band shortens while the A band remains constant.

In the folding model the contractile force is generated in the thin filament and its length is supposed to decrease during shortening. In the sliding model, since the length of both the thick and thin filaments remains the same, the contractile force must stem from mechanical interaction between the filaments.

The common feature of both the folding and sliding contraction mechanisms is *relative motion* between the two sets of filaments. This means that if reactive sites were distributed along both sets of filaments, and if some kind of interaction between thick filament sites and thin filament sites were stoichiometrically linked to the driving chemical reaction, the relative motion of the myofilaments, and therefore of the sites, would influence the kinetics of the chemical reactions. Then the chemical processes supplying energy to the contractile mechanism would be controlled by motion of the muscle. This hypothesis is the

* Whether shortening takes place by *sliding* or *folding* of the thin filament could be decided by the change in the H zone during (not after) contraction. However, the width of the H zone is below the resolving power of the light microscope, so it is difficult to settle this point in experiments with living fibers. I believe that the question is still unresolved; models based on both mechanisms will be discussed.

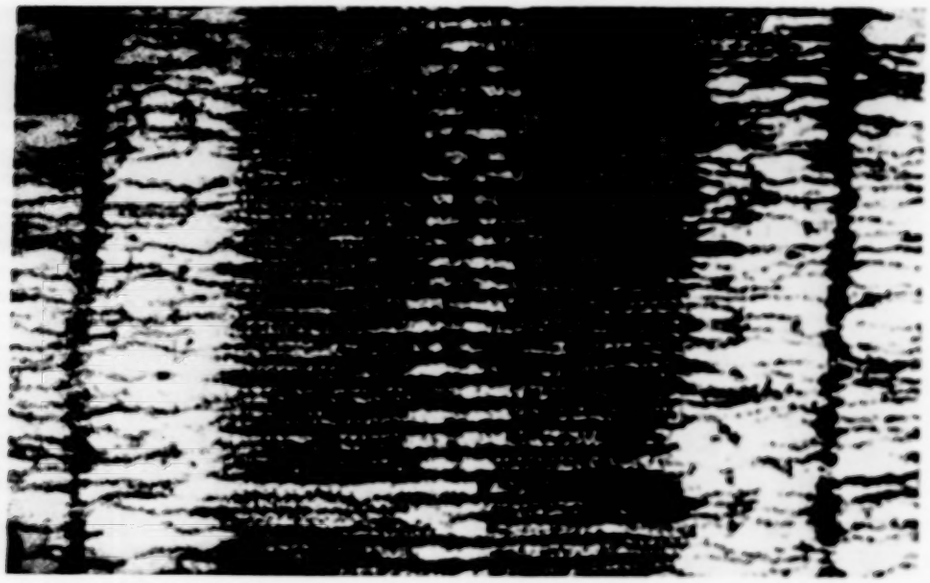

FIG. 8. Double array of filaments in striated muscle. Electron micrograph of longitudinal section of sarcomere (length = 2.5 μ). (From HUXLEY, H. E. and HANSON, J. *J. Biophys. & Biochem. Cytol.*, 3: 361, 1957 [6].)

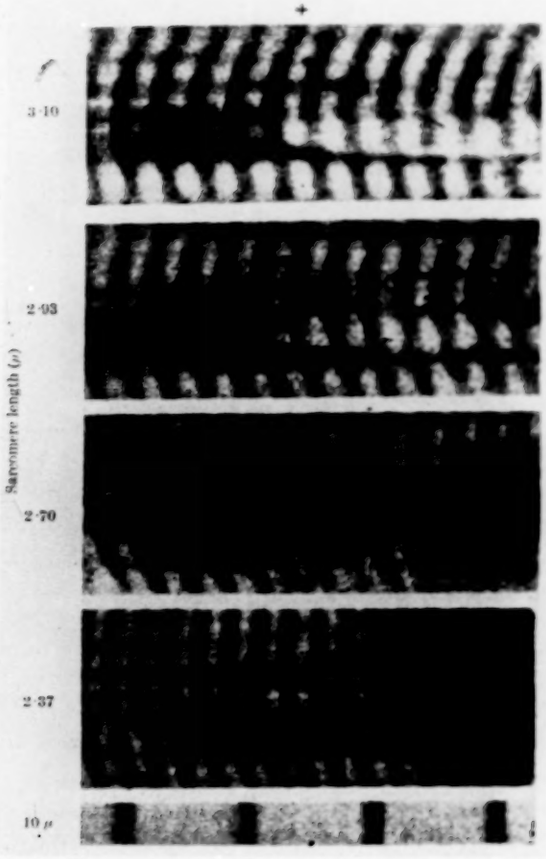

FIG. 9. Contraction of a living muscle fiber. Sarcomere length for each phase of contraction is given on the left. A bands are dark; I bands are light. A bands remain of almost constant width. (From HUXLEY, A. F. and NIEDERGERKE, R. *Nature, London*, 173: 971, 1954 [8].)

basis of several models that quantitatively account for physiology of contraction (that is, for the velocity dependence of both the energy and force output of living muscle).

Implications of Relative Motion. The basic idea is shown in Figure 11. Sites D are distributed along the thick filament and complementary sites K are distributed along the thin filament. Suppose that each (chemical) interaction between D and K sites is associated with the utilization of a substrate molecule, M. A mechanical force generating process may also be associated with this interaction, as in the sliding model, but this is irrelevant as far as the *chemical* kinetics are concerned.

Sites constrained to move on filaments differ from sites on molecules in solution in that the *encounter rate* in the former is function of the relative speed of the filaments while in the latter it depends on the concentration of the carrier molecules. In particular, the encounter rate of sites on moving filaments is a linear function of the relative velocity. (Fig. 12.)

What is the *probability* that a K site will interact with a D site in a given encounter? To some extent this will depend on the specific nature of the interaction. Two kinds of interaction have been examined theoretically [9–11]. One scheme assumes that K always *can* interact with D upon passing, but whether or not it *will* depends on the time K spends in the neighborhood of D: the shorter the time, that is, the higher

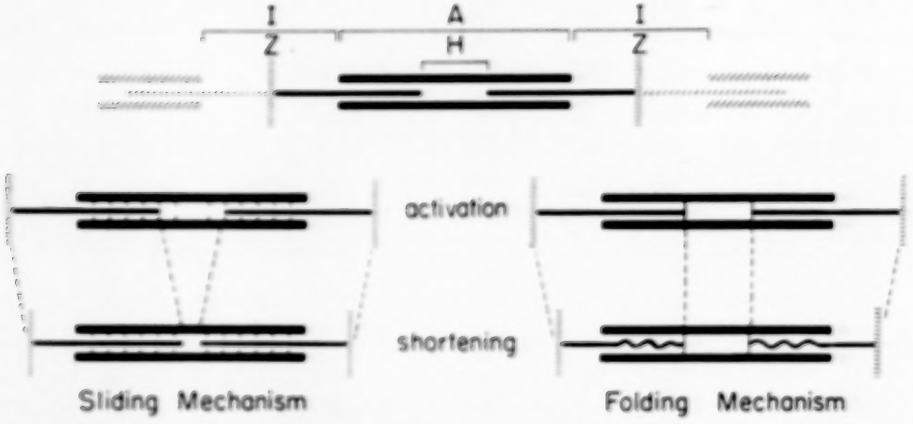

FIG. 10. Hypothetical mechanisms for muscular contraction. *Upper*, the configuration of thick and thin filaments in resting muscle (after Figure 8). *Lower*, change in filament configuration and shape in *sliding* and *folding* contraction models.

the relative speed, the lower the probability will be. Since a substrate molecule M is used up at each K-D interaction, the probability of interaction in a transit of K past D will also be proportional to the energy released for a given amount of shortening.

A physically different scheme visualizes the sequence of events as follows: substrate molecules M are carried by K past D. If K is loaded with M, interaction takes place at D, emptying K, *regardless* of the speed of the encounter. In this case the rate limiting step is assumed to be the filling of K with another M after it has been emptied by D. The filling probability will be lower the shorter the time spent between D sites, that is, the higher the speed. It turns out that the probability factor in this scheme has exactly the same form as in the previous case, where "proximity time" rather than "filling time" is rate limiting [*11*].

The interrupted line in Figure 12 shows how, in *both* schemes, the interaction probability decreases with speed. (This is the Fenn effect.) The number of *successful* interactions per unit of time (solid line, Fig. 12) is the product of the probability of interaction and the encounter rate. Both this function and the rate of driving chemical reaction in living muscle (Fig. 7) depend on the velocity of contraction in the same way. This equivalence can be taken as indirect evidence that the two kinds of filaments seen in the electron microscope do indeed interact chemically at sites along their length, and that these interactions are rate limiting for the chemical reaction that drives the contractile mechanism.

Force. Relative motion of the myofilaments influences the force generated during contraction as well as the rate of chemical reaction. We shall describe two models that quantitatively account for this phenomenon and then show that both of these models can be reduced to the same basic idea.

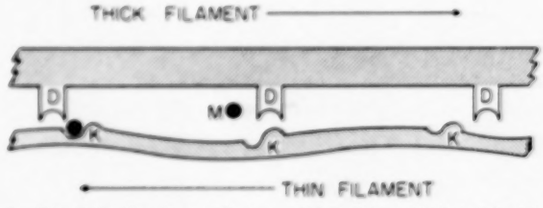

FIG. 11. Distribution of sites along the thick and thin filaments. See text for explanation.

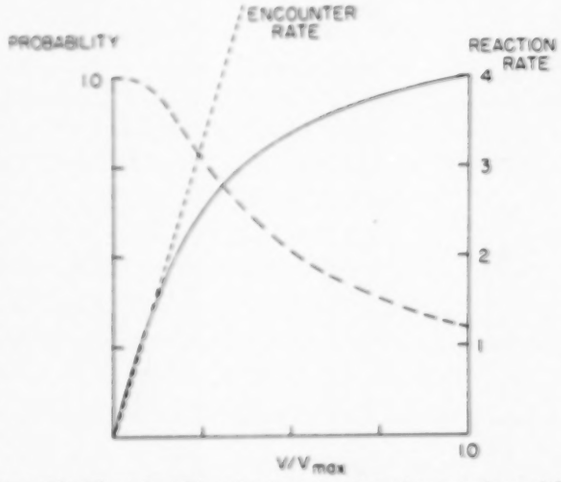

FIG. 12. Chemical kinetics for reaction sites moving with relative velocity, V. *Interrupted line*, probability of interaction (rate limited by proximity time) or of filling (rate limited by filling time); *dotted line*, encounter rate; and *solid line*, reaction rate = (probability) × (encounter rate).

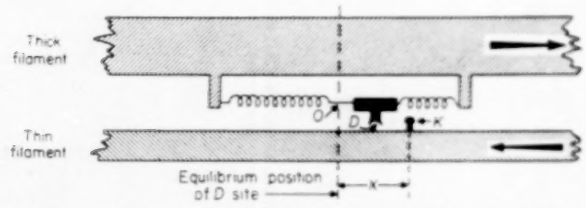

FIG. 13. Mechanochemical element in sliding model by Huxley. x is distance of thin (actin) filament site, K, from equilibrium position of thick (myosin) filament site, D. See text for further explanation. (From HUXLEY, A. F. *Progr. Biophys. & Biophys. Chem.*, 7: 225, 1957 [9].)

A sliding model: The first analysis of a contractile mechanism based on thick and thin filaments in relative motion was made by A. F. Huxley [9]. He assumed a sliding mechanism in which the D site on the thick filament has mechanical properties diagrammed in Figure 13.

D oscillates about its equilibrium position, O. When the K site passes, it can interact with D to form a mechanical connection. The probability of forming a connection in a D-K transit depends on the relative speed; this is an example of interaction in which "proximity time" is rate limiting. Each connection is, in time, broken. The connection is "spontaneous" (it does not require chemical energy); the reverse step, disconnection, requires a substrate molecule, M. Thus one step of the driving chemical reaction (Fig. 6) is associated with each connect-disconnect cycle.

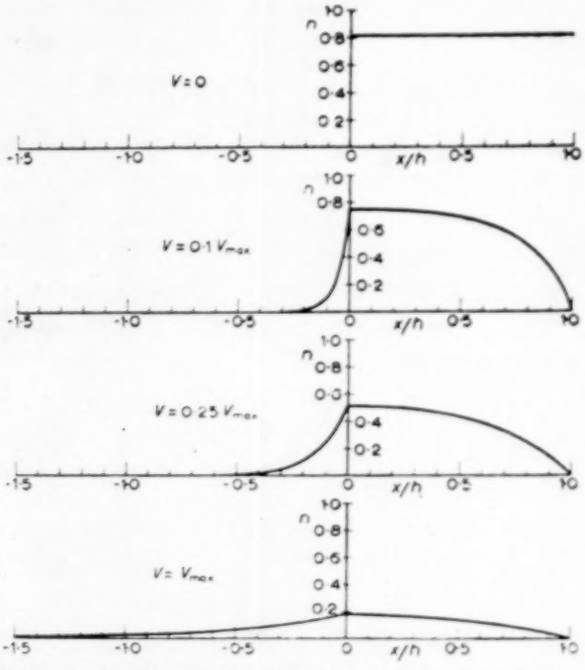

FIG. 14. Distribution of links between thick and thin filaments in steady motion of sliding model by Huxley. n is fraction of D sites at a distance x from the equilibrium position that are connected to K sites; V is relative velocity; h is maximum value of x. (See Fig. 13.) (From HUXLEY, A. F. *Progr. Biophys. & Biophys. Chem.*, 7: 225, 1957 [9].)

If D and K connect, the elastic elements holding D to the thick filament will exert a force on the thin filament which is proportional to the distance of D from O. If the thin filament is moving to the left, connections with D to the right of O will make a positive contribution to the force. If D is still connected to K when it is to the left of O, a force will be exerted which tends to retard the motion.

To insure that the net force exerted by a population of such "pullers" will tend to shorten the muscle, Huxley [9] postulates that D can connect with K only when it is to the right, say, of O. The probability for breaking a connection also depends on x; it is small to the right of O but large to the left. Motion of the thin filament carries connections from the right to the left; when carried past O, the connection tends to retard the motion.

The force developed by the model depends on speed because both the *number* of attachments and their *distribution* about the equilibrium position of the D site depends on speed. (Fig. 14.) When the filaments do not move, the links are all to the right of O; connections can be made only on this side, and there is no motion to carry them to the left. In steady motion, there will be fewer pulling links (since probability of interaction decreases with speed) (Fig. 12), and some links will be carried to retarding positions, so the net force will fall. At the maximum speed, the pulling and retarding forces balance and there is no net force. Huxley [9] showed that these shifts in both number and distribution of connections between filaments with relative speed can quantitatively account for the force-velocity relation in living muscle.

The diagram also shows why there *must* be steady motion for a less than maximum force to remain constant. Consider, for example, the distribution of connections when the speed is one tenth of the maximum. If the motion should suddenly stop, after some time additional pulling links would form to the right of 0, some of the retarding links to the left of 0 would open, and the net force would increase. The original force could be re-established by relaxing the pulling links or stretching the retarding links, that is, by a displacement of the "connection contour" to

the left. This is, of course, what happens when the thin filament slides past the thick filament. A steady force can be set up only when the motion in a given time interval just compensates for the net increase in pulling links formed in that same period.

A folding model: In a folding model [*10,11*] the contractile force is assumed to arise from a change in state of the thin filament. The change in state is brought about by the binding (or reaction) of a substrate molecule, M, to the filament at a K site. Although several mechanisms have been proposed for this process [*12–14*], the essential property is that the force generated by the filament is proportional to the number of occupied K sites. The model is completed by the further assumption that, as the thin filament shortens by folding, force generating molecules, M, are removed from K sites when they pass complementary sites, D, on the thick filament: the reaction $K{\cdot}M \rightarrow K + M'$ is catalyzed by the D site. Then some time passes before K becomes occupied again ($K + M \rightarrow K{\cdot}M$). The latter assumption makes the folding model an example of interaction between thick and thin filament sites in which "filling time" is rate limiting.*

To explain the force-velocity relation, consider Figure 11 again. Suppose the force is a third of the maximum. Then one binding site, K, out of three will be filled; two out of three will be empty. A substrate molecule from the environment will, in time, find its way to one of the empty sites. When the site fills, the force in the filament will rise above that of the load. To reestablish mechanical equilibrium, the filament shortens by folding until one of the filled sites passes a D site, which removes a M, restoring the force to a third of maximum again. The process will be repeated when another binding site is filled. In the steady state, with many sites in action, the rate of motion will be such that the emptying of *full* K sites (passing D sites) is just balanced by the filling of *empty* K sites (between D sites). Since the rate of emptying increases with speed (Fig. 12) and the time required for filling is assumed to be independent of speed, the average occupancy of K sites will be smaller the faster the motion. Now, the average occupancy of K sites is the contractile force; therefore, the force will also be smaller the faster the motion.

The force-velocity relation for the steady state can be worked out explicitly by noticing that ξ, the rate of the driving chemical reaction,* is also equal to the rate of filling K sites. Now, ξ is proportional to the sum of the work flux, PV, and the heat flux, aV, (Fig. 7):

$$\xi = c(P + a)V \qquad (1)$$

where c is a proportionality constant. The rate of filling K sites depends on the number that are empty which, in turn, is proportional to $(P_0 - P)$. Therefore, in the steady state,

$$(P + a)V = b(P_0 - P) \qquad (2)$$

where b is a proportionality constant. Equation (2) is Hill's expression [*2*] for the force-velocity relation.

Under a given set of experimental conditions, a is constant and proportional to P_0. Therefore, the maximum speed, $V_{max} = (P_0/a)b$, is proportional to b. We shall return to these considerations when we consider the contractility of muscle.

The Length-Tension Relation. The magnitude of P_0, the isometric tension depends on muscle length;† in skeletal muscle, P_0 is a maximum at normal body length [*15*]. Although the basis of the length-tension relation is not clear, the change in relative position of thick and thin filaments with sarcomere length provides some clues. (Figs. 8 and 10.)

For example, the decrease of P_0 at the shorter muscle lengths is correlated with the disappearance of the H zone. When this happens the ends of the *thin* filaments (which define the H zone) meet and butt against each other. This could impede further sliding of the thin filaments in the sliding model. The result would be the same if shortening were a consequence of thin filament folding: as in a spring or a rubber band, the force generated by the filament (at a given occupancy of K sites) would be smaller, the shorter the filament.

* This folding model resembles the viscoelastic model insofar as force is generated by an elastic filament. However it differs from the viscoelastic model because force is controlled by *chemical* interaction between molecules on the elastic (thin) filament and its environment (sites on the thick filament) rather than by *physical* (viscous) interaction between the elastic filament and its environment.

* ξ is the velocity dependent component of the total reaction rate, ξ_T. Therefore, $\xi = \xi_T - \xi_M$, where ξ_M is the velocity independent component of the reaction rate. ξ_M provides the maintenance heat.

† In our discussion of the force-velocity relation we assumed that the change in length during contraction was sufficiently small (<10 per cent) that changes in P_0 could be neglected. Contractions of skeletal muscle in the body rarely exceed this limit.

The contractile force is nearly zero when the I band closes up. Presumably the ends of the *thick* filaments butt against each other at these lengths, preventing further shortening.

The decrease of P_0 at the longer muscle lengths can be understood if force is developed only by that part of the thin filament which interdigitates with thick filaments. This is an obvious consequence of the sliding model; in the folding model, it implies that substrate molecules, M, are available only in the A band.

The shortening ability of the contractile mechanism is lost when the sarcomere is stretched to a length where the thick and thin filaments no longer overlap [*16*]. Shortening ability reappears when the sarcomere is shortened slightly, restoring filament overlap. This also follows from the models because mechanical continuity along the fiber axis is necessary for contraction. In the sliding model, mechanical connections between thick and thin filaments are made along the entire overlap region; in the folding model, however, it is assumed that only the *ends* of the thin filament bond to adjacent thick filaments when the muscle is activated. (Fig. 10.)

Generalized Mechanochemical Model of the Contractile Mechanism. Both the sliding model worked out by Huxley [*9*] and the folding model I have examined [*10,11*] have the same basic structure:

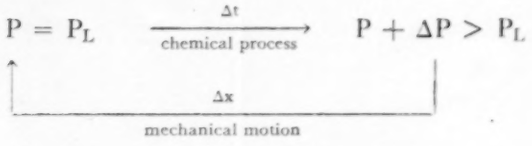

FIG. 15. The mechanochemical cycle.

The models (and presumably living muscle) can be represented by a *mechanochemical cycle* with the following properties:

(1) contractile force is increased by ΔP in time Δt;
(2) contractile force is decreased by $f\Delta x$ in shortening Δx;
(3) contractile force is continuously balanced against the load, P_L.

In (2) f is the decrease in force when $\Delta x = 1$ and $\Delta t = 0$.

The cycle operates in the following way: suppose the contractile force is less than full isometric value, P_0. Then chemical reactions take place which *increase* the force (in the sliding model, pulling connections are made; in the folding model, empty binding sites are filled). Thus, if the system is initially mechanically balanced (the force generated by the contractile mechanism, P, equals the load, P_L), after time Δt the contractile force will exceed the load by an amount ΔP. Since shortening *decreases* the contractile force (in the sliding model, pulling connections are relaxed and retarding connections are stretched; in the folding model, full binding sites are emptied), the mechanical unbalance can be righted by shortening an amount Δx. The contraction velocity, V, necessary to maintain the force at P_L is $\Delta x/\Delta t$. The relation between V and a steady force is, of course, given by the force-velocity relation. (Fig. 3, equation 2.)

In isometric contraction, the *length* of the muscle, rather than the external load, is kept constant. Then the contractile mechanism shortens against the *series compliance* of the myofilaments, the tendons, the recording apparatus, etc. In this case the following sequence of events takes place: when the muscle is activated, the initial load is the unstretched series compliance: $P_L = 0$. After time Δt_1, the contractile element has developed force ΔP_1. To balance the forces, the contractile element shortens an amount Δx_1 (thereby losing force $f\Delta x_1$), stretching the series compliance by the same amount. This *internal* motion stops when the force in the contractile element ($\Delta P_1 - f\Delta x_1$) equals the force in the series compliance ($k\Delta x_1$); k is the elastic coefficient of the series compliance. In the next element of time, Δt_2, the chemical process develops additional force, ΔP_2. Again the contractile element will shorten an amount Δx_2 at the expense of the series compliance, balancing the forces: the force at the end of the muscle is now $k(\Delta x_1 + \Delta x_2)$. This cycle repeats until either the substrate for the chemical process runs out or the total force reaches P_0. The latter is the case in tetanically stimulated skeletal muscle; the former happens in a twitch or a heart beat.

The rate of force development, $\Delta P/\Delta t$, in the chemical part of the cycle depends on several factors. In the first place, $\Delta P/\Delta t$ depends on P; $\Delta P/\Delta t = 0$ when $P = P_0$, then $V = 0$; $\Delta P/\Delta t$ is a maximum when $P = 0$, then $V = V_{max}$. For a given value of P, $\Delta P/\Delta t$ also depends on the *absolute rate* of the chemical process. In particular, for $P = 0$, $\Delta P/\Delta t$ — and consequently V_{max} — will be proportional to the absolute rate of the chemi-

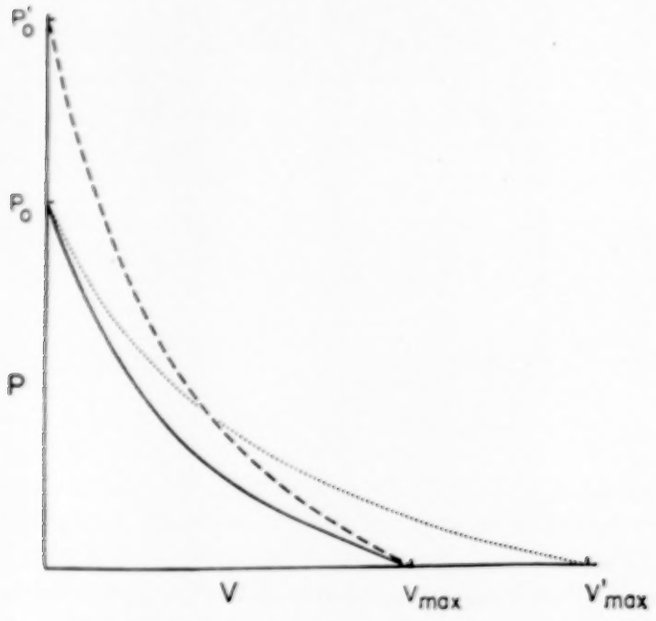

FIG. 16. The force-velocity relation and contractility. *Solid line*, reference state characterized by P_0 and V_{max}; *dotted line*, $V_{max} \rightarrow V'_{max}$, and *interrupted line*, $P_0 \rightarrow P'_0$.

cal process. Turned around, this means that V_{max} *is an experimental measure of the absolute rate of the force generating chemical process.* Factors that increase this rate (reactant concentration, for example) will increase the value of V_{max} to V'_{max}, and there will be a corresponding change in the force-velocity relation. This is illustrated in Figure 16 by the change of the force-velocity relation from the solid line to the dotted line. In skeletal muscle, increasing temperature accelerates the absolute rate of the force generating chemical process and shifts the force-velocity curve in just this way [*2*].

The mechanical part of the cycle is characterized by the value of f, the decrease in force per unit distance when the contractile mechanism shortens very rapidly, that is, at speeds so fast that the chemical process in the cycle can be neglected. Once the chemical process is outpaced (which will be the case when $V \gg V_{max}$) the fall in force with distance is independent of shortening speed. Since this is analogous to the relaxation of a stretched elastic element, f is the elastic coefficient of the "*intrinsic elasticity*" of the contractile mechanism.* (In the sliding model, "intrinsic elasticity" resides in the elastic connections between thick and thin filaments; f is the elastic coefficient of the spring in Figure 13.

* The elastic coefficient of the "series elastic element" of the muscle, defined by Hill [*17*], is $fk/(f + k)$.

In the folding model, "intrinsic elasticity" reflects the fall in force as M is removed from the thin filament when, in the course of shortening, full thin filament sites, K·M, encounter thick filament sites, D.) Since the intrinsic elasticity depends on physical properties of structural elements of the contractile mechanism, it is relatively insensitive to environmental factors.

Activation of the Mechanochemical Cycle. When a muscle fiber is depolarized by an action potential, a limited amount of substrate (M) is made available to the contractile mechanism. The mechanochemical cycle then begins to turn over and continues to do so until the supply of M is used up. The mechanical response depends primarily on the supply of M; however, for a given amount of M, the myogram depends on both the speed of the chemical process and, since substrate utilization increases with velocity (Fig. 7), on the mechanical loading.

Environmental factors influence the length of time substrate is made available to the mechanochemical cycle after the action potential. For example, in skeletal muscle the twitch is enhanced when nitrate is substituted for chloride in the Ringer solution: both duration and peak tension increase [*18*]. (Fig. 17.) The isometric tension rises at the same rate as in chloride (left, Fig. 17) and the isotonic contraction shortens at the same speed. (Right, Fig. 17.) This means

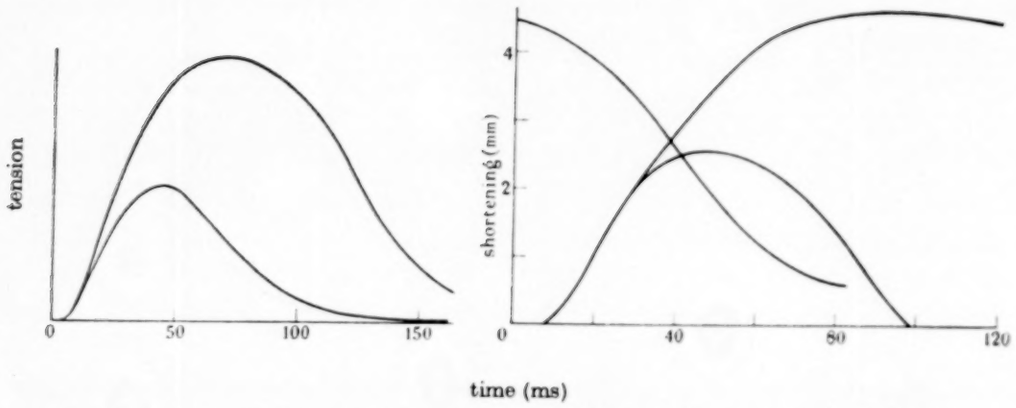

FIG. 17. Twitches of the sartorius muscle of the frog in chloride- and in nitrate-Ringer solution. *Left*, two isometric twitches: the lower in chloride-Ringer, the upper in nitrate-Ringer, 18°C. *Right*, two isotonic twitches, without afterload: the lower in chloride-Ringer, the upper in nitrate-Ringer, 18°C. Note that the nitrate twitch continues in a second sweep of the oscilloscope. (From HILL, A. V. and MACPHERSON, L. *Proc. Roy. Soc. London, s.B.*, 143: 81, 1954 [*18*].)

that in both chloride and nitrate Ringer the mechanochemical cycle operates along the *same* force-velocity curve; in nitrate, since it operates longer, substrate must have been available for a longer time. Although the mechanical response changes, the *contractility* of the muscle (measured by P_0 and V_{max}) is unaltered.

In contrast, in heart muscle, contractility depends on the chemical background.* Experimentally, this is reflected in changes in the force-velocity relation.

One set of conditions (for example, increased concentration of calcium) seems to increase P_0 to P_0', which multiplies the ordinates of the force-velocity relation by P_0'/P_0 [*19*]. (Interrupted line, Fig. 16.) Under these conditions the myogram has the same duration as "normal" but the amplitude is multiplied by the ratio P_0'/P_0. (b, Fig. 18.) This implies that a greater number of tension generating sites have become available in the sarcomere. One explanation is that the tension generating capacity of the mechanochemical cycle *per se* has been increased. An alternative explanation is that the *number* of mechanochemical cycles (activated myofilaments) has been increased. In other words, after depolarization under normal conditions, only a fraction of the myofilaments are activated; when calcium concentration is increased, a greater number of myofilaments (all having the *same* mechanochemical properties) become activated. In this case, muscle contractility is enhanced simply because a greater number of mechanochemical elements operate in parallel.

A second set of conditions (for example, addition of norepinephrine) seems to increase the absolute rate of the chemical process in the mechanochemical cycle. This is reflected as an increase in the V_{max} of the muscle and a change in the force-velocity relation [*19*]. (Dotted line, Fig. 16.) In the myogram, the tension rises more rapidly than normal and peaks earlier. (c, Fig. 17.)

* I am indebted to Dr. E. H. Sonnenblick of the National Heart Institute, Bethesda, Maryland, for rewarding discussions about heart muscle and for making available, prior to publication, the results of his study of the papillary muscle of the cat.

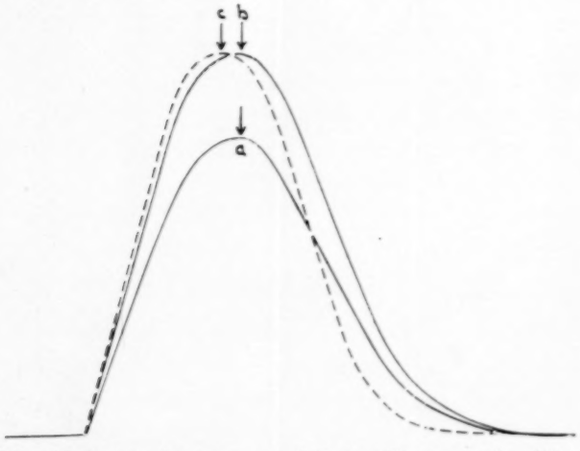

FIG. 18. Isometric myogram of the papillary muscle of the cat (diagrammatic). Maximal stimulation at constant rate in Krebs-Ringer, 27°C. (a) Ca = 2.5 mM; (b) Ca = 5.0 mM; (c) Ca = 2.5 mM + norepinephrine. Peak tension in (a) and (b) occurs at nearly the same time; peak tension in (c) occurs earlier. (From SONNENBLICK, E. H., unpublished experiment.)

In summary, activation of the contractile mechanism by an action potential allows the chemical process in the mechanochemical cycle to proceed to a limited extent. A convenient characterization of the operation of the cycle is the force-velocity relation: it defines the contractility of the muscle. The contractility of heart muscle is more sensitive to chemical background than that of skeletal muscle; this gives the heart more flexibility in handling its affairs.

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Some Observations and Theories Concerning the Electrical Behavior of Heart Muscle*

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"How in words can you describe this heart without filling a whole book? Yet the more details you write concerning it, the more you will confuse the mind of the reader."

LEONARDO DA VINCI [60]

ELECTROPHYSIOLOGY of heart muscle may be considered the science of the nature and propagation of electrical energy produced by heart muscle fibers submerged in a conducting medium. It deals with the generator and the medium but, at least for the purpose of this review, not with the problems of lead fields, lead placement, lead systems, or with the representation of projected cardiac electromotive forces on the body surface. It forms a basis, as yet incomplete, for a rational interpretation of an electrocardiographic curve or vectocardiographic figure. This survey is intended merely to highlight general principles and certain interrelationships. It will be concerned with (1) the basic electrical properties of heart muscle fibers, (2) the quantity and distribution of electrical forces over cardiac musculature, and (3) the distribution of electrical forces in volume conductors. Recent monographs should be consulted for more detailed discussions [1–10].

ELECTRICAL PROPERTIES OF CARDIAC FIBERS. *Some physiological aspects of subcellular structure:* The cardiac cell is excitable tissue, i.e., upon appropriate stimulus a transient change in the physicochemical properties of the cell occurs permitting alterations in ionic transfer across its boundary. The effect is usually all or none, and the disturbance is generally propagated from cell to cell. Heart muscle shares this property with nerve tissue, skeletal and smooth muscle. There are certain fundamental similarities between the electrical behavior of all excitable cells so that information gained from one can be applied to the others. There are also important dissimilarities, apparently related to the specific function of individual tissues. Since most information concerning the cellular events of excitable systems so far has been derived from nerve cells, the laws which define electrical behavior in nervous tissue are used as the prototype. Such cells resemble cables or telephone wires with one long (infinite) axis and one of relatively short diameter. For many years the "cable theory" of electrical conduction developed by Thomson (Lord Kelvin) in 1856 has been applied to biologic systems of this general kind [14,27]. The short, thick, grossly syncytial heart muscle fiber is a structure of greater complexity than nerve. This modifies some of the assumptions made from the study of nerve cells.

Whether or not heart muscle can be considered truly syncytial in nature depends on one's point of view. Heart muscle fibers are interrupted by light-refractile bands which traverse the fiber from fibril to fibril. The function and properties of these "intercalated discs" are considered by some to be consistent with the existence of an absolute cell boundary [89]. They are the starting and terminal points of insertion of the myofibrils, and in a mechanical sense these structures can be considered boundaries. Electrical resistance across the discs, however, is not high [8], which may be accepted as evidence in favor of at least a functional syncytium. There are differences in electrical responses of heart muscle fibers from different species, perhaps simply related to the modifying effects of temperature and heart rate, and there are qualitative and quantitative differences in the electrical behavior of various areas within a single heart. These differences should have their morphologic counterpart. As yet little is known of submicroscopic details of cardiac tissue on which

* From the Department of Medicine, University of Utah College of Medicine, Salt Lake City, Utah. This study was aided in part by grants from the Utah Heart Association and the U. S. Public Health Service grants HT-250 and HTS-5150.

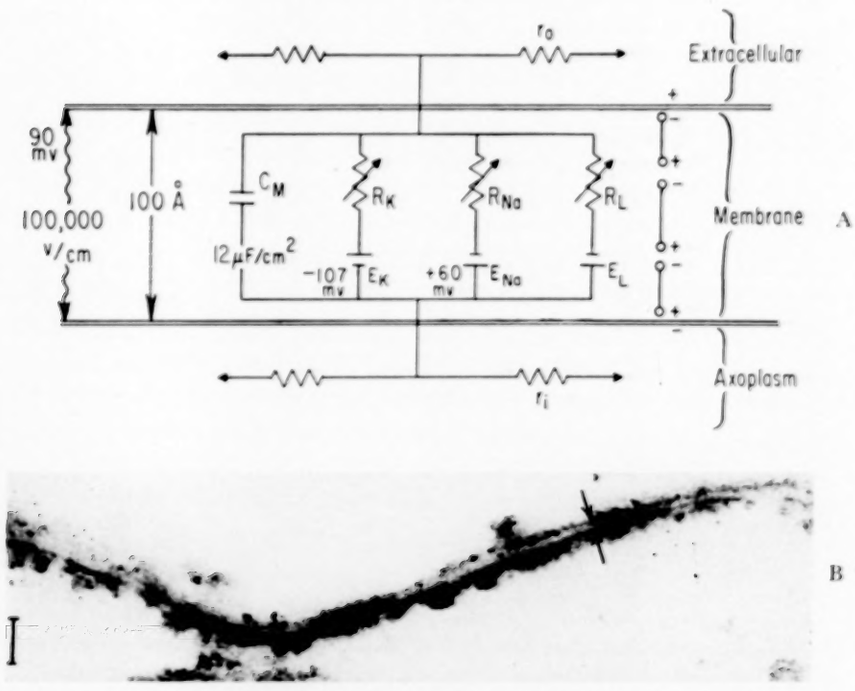


FIG. 1. A, electrical circuit model of a unit of excitable tissue. The membrane is "polarized" (oriented molecules at the right). C_M membrane capacitance, R_K, R_{Na}, and R_L resistance to passage of K^+, Na^+, and other ions respectively, E_K, E_{Na} and E_L "battery" for K^+, Na^+, and other ions, r_o and r_i resistance of extracellular fluid and axoplasm. The resistance to the passage of ions is variable depending on the state of excitation. The sum of the resistance values is R_M of Figure 2. The values listed are those assumed or calculated for heart muscle. B, electron microscopic picture of an actual "membrane" showing a double layer (from Robertson [*83*]). Orientation as in A. Arrows point to extracellular and intracellular boundary of "membrane." Scale 0.1 μ times 96,000.

these electrical differences might depend. As in other tissues, a boundary (membrane) separates intra- from extracellular cardiac compartments and, indeed, a double-layered membrane can be seen in an osmium-stained electron microscopic preparation. (Fig. 1.) The presence of such a boundary, not necessarily coinciding with the histologic cell membrane, is essential for the maintenance of the excitatory state. For heart muscle, its properties have been well studied. In general, such electrical boundaries (membranes) are double-layered, 100 Å (10^{-7} mm.) thick, and consist of a few layers of lipoprotein molecules which are capable of orientation in a strong electric field (polarization). At rest such a membrane maintains a charge of 100,000 volts/cm. Other physical-chemical properties of this structure in heart muscle have been summarized recently [*48*].

The microelectrode and microelectrode recording: The electrical properties of single cardiac fibers have been elucidated by measurements with a recording system employing as an exploring electrode a glass capillary with an external tip diameter less than 1 μ (0.001 mm.). The tip of the capillary is filled by boiling with 3 M potassium chloride solution, which approximates the intracellular milieu and avoids significant electrochemical potentials at the boundary of electrode and cytoplasm (junction potentials) [*36,73*]. A tungsten wire (to avoid polarizing effects) connects the electrolyte solution within the capillary to the closely adjacent first stage of the recording device. Another tungsten wire serves as the indifferent electrode in the bathing solution, or for *in situ* experiments is placed on or in the body at a distance from the heart. The glass capillary is mounted on a micromanipulator and is made to penetrate the protoplasmic boundary. It records variations in electrical potentials between its location within the single fiber and the extracellular surrounding. Because

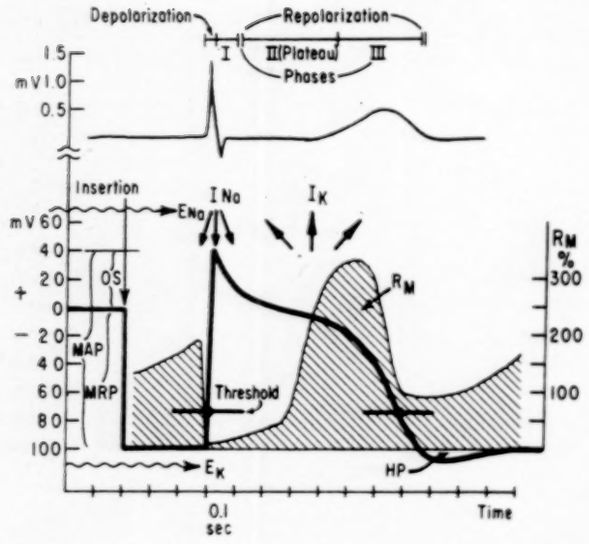


FIG. 2. Cardiac excitation. A *surface electrocardiogram* (above) and *transmembrane potential* of a cardiac muscle fiber (below). A microelectrode on penetrating the fiber (insertion) records a large negative potential (MRP = membrane resting potential). On excitation (depolarization) the interior of the fiber becomes positive and the tracing moves above the isoelectric line. OS = overshoot, this defines the positive component of the transmembrane potential. MAP = membrane action potential, constitutes MRP + OS. Repolarization occurs slowly during RS-T segment and T wave, and at least in ventricular muscle several "phases" of recovery can be recognized [*110*]. HP = hyperpolarization after recovery. "Threshold" signals the level of MRP at which spontaneous depolarization will occur. RM, in relative units, indicates fluctuation in transmembrane resistance (after Weidmann [*9*]). I_{Na} and I_K the approximate times for maximum sodium inward and potassium outward currents. E_K and E_{Na} approximate values for potassium and sodium equilibrium potentials (see text).

of their small tip size such microelectrodes have a high resistance despite the concentrated potassium chloride solution with which they are filled. This needle resistance varies between 5 and 30 MΩ, requiring therefore a high input resistance of the recording device.

If R_E represents electrode resistance, R_A input resistance of the recorder, and E the voltage to be obtained, the actual voltage E′ recorded may be calculated by

$$E' = E \frac{R_A}{R_E + R_A} \qquad (1)$$

Even if R_A, the input resistance of the amplifier, is ten times the value of R_E, the recorded potential E′ will be only E × 0.91 [*61*]. Input resistance of the instrument must therefore be at least 10^{10} MΩ in order to measure the applied voltage accurately. The classic device used as the first stage of the recorder is the cathode follower which has no appreciable gain but is characterized by a high input and a low output impedance. Such high instrument resistance values, however, delay the response of the entire system to a rapidly changing applied voltage; the rise time, the time required to record 90 per cent of the applied voltage $\left(\frac{dV}{dt}\right)$, in the microelectrode assembly is usually between 30 and 100 microseconds (μsec.). This is generally sufficient for events occurring at the cardiac fiber. The use of a string galvanometer instead of the oscilloscope as the display unit will further magnify this deficiency with a rise time of the galvanometer of from 5 to 20 milliseconds (msec.). Records so obtained can not therefore estimate the rapid changes which occur during the beginning of cardiac excitation.

Cellular resting and action potential: If properly inserted into the fiber interior the membrane will seal itself around the electrode, preventing escape of cytoplasm. After inserting a second electrode into the same fiber a decrease in potential can be recorded by the first electrode, apparently caused by insertion of the second. For large fibers this amounts to no more than 1 to 2 millivolts (mv.) [*36,73*]. On penetrating the membrane a transmembrane potential is recorded which for heart muscle fibers varies from 50 to 100 mv., cell interior negative to cell exterior. (Fig. 2.) The values of this *resting potential* depend to some extent on the technic of impalement. Large fibers (easier to penetrate) yield higher values than small structures. Purkinje fibers have the largest resting potentials, atrial tissue and particularly the cells of the sinus region have low values. (Table 1.) It is likely that these differences are real and not merely a function of the impalement.

Each heart beat (excitation) is associated with a striking change in potential occurring within each fiber: the large negative diastolic potential falls abruptly to zero and beyond. It "overshoots" so that at the height of excitation the cell interior is positive, the outside negative. (Fig. 2.) Although this reversal of potential was noted very early [*21a*] the process of excitation was generally thought to consist simply of removal of charges (depolarization) leaving no potential difference, no physiological cellular boundary within the excited region. This was Bernstein's original theory [*11*]. While Bernstein's notion of

TABLE 1
CARDIAC ACTION POTENTIAL

Fiber	Species	Diameter of Fiber (μ)	MRP (mv.)	MAP (mv.)	APd (msec.)
Heart					
Sinus node	Frog	5	40	53	200
	Turtle	...	36	42	...
	Rabbit	...	60	66	200
Atrioventricular node	Dog	...	53	58	350
Atrium	Frog	5	70	90	400
	Turtle	5	56	65	600
	Rat	10	62	75	50
	Chick (embryo)	(5)	30	40	50
	Rabbit	...	78	92	150
	Cat	10	60	65	250
	Dog	10	85	105	200
Ventricle	Frog	10	80	95	600
	Turtle	...	85	110	900
	Rat	...	90	120	...
	Chick (embryo)	(5)	40	54	200
	Rabbit	...	80	102	...
	Cat	16	80	100	400
	Dog	16	80	100	400
	Man	16	65	95	200
Purkinje fiber	Cat	16	88	116	100
	Dog	30	90	120	300
	Sheep, calf	75	94	130	400
Comparative values					
Unmyelineated nerve	Squid	400	65	85	0.4
	Lobster	75	65	110	0.7
Myelinated nerve	Frog	15	70	115	...
Skeletal muscle	Frog	137	88	123	1.5

NOTE: MRP = membrane resting potential; MAP = membrane action potential; APd = action potential duration. These data are average values depending on heart rate and temperature [*4,8,9,35,52,88*].

the resting state has remained valid, his concept of excitation has been invalidated by newer experimental facts.

The overshoot quickly decreases after reaching a maximum value but complete restitution of the resting state in heart muscle is a much more gradual process which appears almost arrested near the isoelectric line (the "plateau"). Only at the end of systole, and coinciding with the T wave of the electrocardiogram, does a rapid return to the resting level finally occur. In Purkinje fibers, this final return may again overshoot its mark in the opposite direction, so that for a brief period at the end of systole the maximum diastolic potential may exceed the average resting potential (positive after potential). (Fig. 2.) In ventricular muscle this brief hyperpolarization is absent but has been noted occasionally in abnormal states [*2,5,8,10*]. At times hypopolarization may be observed (negative after potential) when after recovery the potential once more approaches the zero line. These afterpotentials have some characteristics of damped oscillations, and if they reach threshold they may play a part in repetitive responses, extrasystoles, or "re-entry" (*vide infra*) [*8*].

If the molecular layers within the membrane are considered strongly orientated in the electric field existing at rest, the breakdown of this field on excitation causes these layers to become "depolarized" with random molecular distribution. While a weak field of opposite sign is present for a brief period at the height of excitation, the membrane may be considered to show "polarization reversal." The exceedingly gradual process of "repolarization" is a characteristic of heart muscle not shared by other excitable tissues in their natural state with the exception of smooth musculature. The sequence of these events is of course rate- and temperature-dependent [*95,96,110*]. The different slopes during the process of repolarization, in particular,

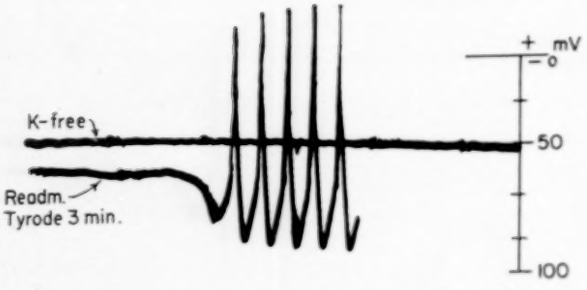


FIG. 3. Action potential of Purkinje fiber of sheep in K^+ free Tyrode solution and after readmission (Weidmann and Hecht, unpublished). Note decrease in membrane potential on K^+ depletion with cessation of spontaneous activity. Restoration of activity coincident with increase in membrane potential. Note also (1) pacemaker activity indicated by diastolic depolarization, (2) dependence of size of overshoot on level of membrane potential (compare first with fourth beat).

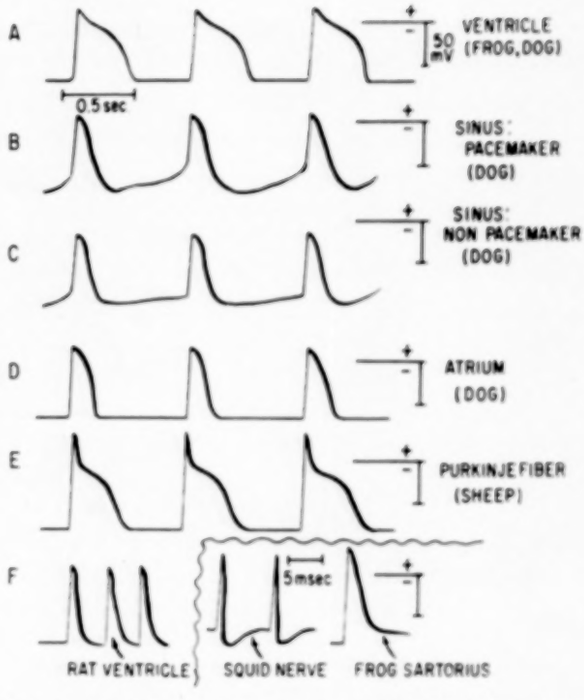


FIG. 4. Various cardiac action potentials with action potentials of nerve and skeletal muscle for comparison.

have been shown to have different temperature coefficients [*110*]. The rate of depolarization and the positive overshoot are clearly dependent on the level of the resting potential [*9*]. (Fig. 3.)

If into a resting fiber a second electrode is inserted through which a current can be passed, the resting potential can be altered passively, either increased (anodal current) or decreased (cathodal current). Passive cathodal depolarization can be carried only to a certain level, usually about 80 per cent of the full value. When brought to this point the cell will spontaneously and rapidly depolarize; it "fires" as an all-or-none response. This *cellular threshold* for firing lies at about 70 mv. for ventricular fibers. (Fig. 2.) Conversely, an anodal polarizing current applied at the height of the plateau will, if strong enough, turn the excitatory process off and return the cell to the resting level [*9,100*]. Therefore, repolarization also can be induced as an all-or-none response at a definite threshold, a phenomenon that has been demonstrated in addition to heart muscle for unmyelinated nerve fibers of the squid [*52*] and for myelinated nerves [*56*]. These observations suggest that spontaneous recovery in heart muscle may at times be a propagated event [*23*] instead of the automatic consequence of cellular depolarization. Since the propagation of this anodal response is slow, normal non-conducted repolarization is likely to overtake this response at some distance from its origin. It may, however, be in part responsible for local events.

Two additional sets of observations require some comment: The general configuration of heart fiber potentials is similar in all species and in all parts of the heart. As mentioned, however, characteristic minor differences exist, striking enough to allow identification of the area from which it was obtained. Several characteristic heart cell potentials are illustrated in Figure 4. In addition, although prolonged depolarization is the hallmark of cardiac action potential, the plateau can be readily abolished under a variety of circumstances [*8,48*] independent of rate and temperature. Such records will then resemble nerve and skeletal muscle potentials. (Fig. 5.) Conversely, intracellular injection of TEA [*93*], increasing external sodium concentration [*66*], or other changes [*90*] may cause prolonged repolarization in nerve cells. Even under "normal" experimental circumstances, medullary nerve fibers show prolonged repolarization time [*91*]. The plateau, therefore, can only be considered a relative characteristic of cardiac muscle.

Electric constants of the heart: The electrical characteristics of heart muscle have been established by Weidmann [*101*] following closely the procedures used by Cole [*25,26*] and Hodgkin [*4,50*]. As stated before, it has been assumed that excitable fibers have some of the characteristics of electrical cables with a well conducting interior, high transmembrane resistance and high

capacity—the ability to hold electrical charges. The equations of cable theory, however, apply to a fiber of uniform diameter and "infinite" length. These are not applicable to the interlacing cardiac fibers. The few measurements available for cardiac muscle are based on the non-branching strands of Purkinje fibers, the best preparation from this point of view. Still, the electrical constants for heart muscle and their possible changes during excitation are fully as important for the understanding of cardiac function as the more familiar parameters of stroke volume, diastolic fiber length, valve circumference, or similar physiologic or morphologic data. The parameters of interest are those of *resistance* across the membrane and along the core of the fiber (and its inverse: conductance), membrane *capacity* (and the inverse: admittance), and membrane *current*. (Table II.)

Membrane resistance: (r_m) "friction." If a current is passed through the interior of an impaled fiber, the intracellular voltage (membrane potential) is changed in proportion to the current applied and the amplitude of the recorded potentials squared gives relative values for membrane resistance [*49b*]. The induced change in potential (electrotonus) spreads through the interior but it declines exponentially as it traverses the length of the fiber and falls to 1/e of its original value at a definite distance [*49a*]. This is a characteristic for each tissue, the "space constant" λ which is expressed as follows:

$$\lambda = \sqrt{\frac{r_m}{r_i}} \qquad (2)$$

where r_i represents the resistance of the cytoplasm per unit length (in Ohms) and can be

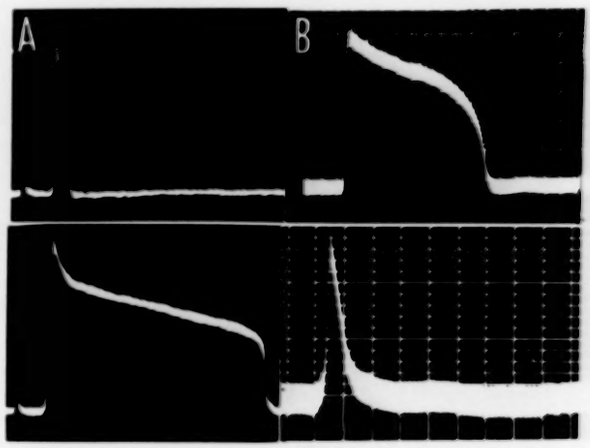


FIG. 5. A, action potential of myelinated nerve of toad before (upper) and after (lower) application of Ni Cl_2 (from Spyropoulos and Brady [*90*]) (duration of lower tracing 13 msec.). B, action potential of ventricular myocardial fiber of bull frog *in situ* before (upper) and after (lower) intracardiac injection of 1.2 mg./kg. digitoxin (from Hecht [*48*]). Time lines 100 msec. Note that the plateau of the repolarization process is only a relative characteristic of heart muscle (see text).

measured. r_i is given by Ohm's law as

$$r_i = \frac{V_o}{I_o} \cdot \frac{1}{\lambda} \qquad (3)$$

where V_o is the potential existing at the current electrode, I_o the current applied to the cell interior. From these measurements r_m, the membrane resistance times unit length, can be obtained. The specific resistances R_m and R_i, also termed *resistivity*, or resistance times unit volume are then given by

$$R_m = \pi a^2 r_m \text{ (in } \Omega\text{cm.}^2) \qquad (4)$$

$$R_i = \pi a r_i \text{ (in } \Omega\text{cm.)} \qquad (5)$$

where a is the radius of the fiber. Table II lists

TABLE II

ELECTRICAL CONSTANTS OF EXCITABLE TISSUES*

Fiber	Species	Diameter of Fiber (μ)	C_M (μF/cm^2.)	λ (mm.)	θ (M./sec.)	R_m (Ωcm^2.)		Ratio (%)		E_M (mv.)	E_K (mv.)	E_{Na} (mv.)
						Rest	Excitation	Rest	Excitation			
Heart												
Purkinje fiber	Kid	75	12	1.9	2.2	2,000	20	100	1	95	−105	+65
Atrium	Dog	10	30	0.4	0.1	280	...	...	...	70	...	...
Skeletal muscle	Frog	137	8	2.4	1.4	4,000	40	100	1	90	−95	+45
Unmyelinated nerve	Squid	400	1.2	2.3	19.0	1,500	25	40	1	65	−90	+50
	Lobster	75	1.3	1.6	...	2,300	...	...	...	65	...	...
Myelinated nerve	Frog	15	0.005	...	25.0	100,000	...	...	...	70	...	...

NOTE: See text for symbols.

*[*4,7,8,9,27,35,50,52,59,73,88,101*].

TABLE III
CONDUCTION VELOCITY, MAMMALIAN HEART MUSCLE*

Fiber	Species	Diameter of Fiber (μ)	E_m (mv.)	D_t (v./sec.)	θ (M./sec.)
Atrioventricular node	Dog	(5)	53	...	0.05
Atrium	Cat	10	60	11.6	0.3
Ventricle	Dog	16	80	150.0	0.9
	Cat	16	80	100.0	1.0
Conduction system	Sheep, calf	75	94	800.0	2.2
	Dog	30	90	610.0	2.0

NOTE: E_m = resting membrane potential; D_t = maximum rate of depolarization; θ = conduction velocity.
* [4,7,8,9,27,35,50,52,59,73,88,101].

these constants for several tissues. In the resting preparation R_m for Purkinje fibers measures 2,000 Ω cm.², λ = 2 mm. It is likely that λ changes depending on the direction of the current flow in the fiber. In rat atrium the value for λ is 50 per cent greater if current flows in the direction of the fiber than transversely across it [33]. The electrical resistance fluctuates widely during the cardiac cycle, very low values indicating high conductance during the crest of excitation [9]. (Fig. 2.) This must obviously be the case if a current is to flow through the membrane on excitation in order to initiate a propagated response over the fibers as the immediate precursor to mechanical contraction. In other words, one cannot expect the heart to beat unless there are local changes in transcellular impedance during the cardiac cycle—a statement which illustrates the importance of these constants as primary factors of heart action.

Membrane capacitance: (C_m; in μ F/cm.²) This is defined as the ability of the boundary to act as a condenser by accepting and later releasing electrical charges (in coulombs). C_m is determined by the equation

$$C_m = \frac{\tau_m}{R_m} \qquad (6)$$

where τ_m is defined as the "membrane time constant." This is determined from the rate of the change in membrane voltage resulting from the application of a constant current step. The membrane capacity of the Purkinje fiber measures 10 to 12 μ F/cm.² [101]. This value is in excess of those obtained for nerve and skeletal muscle, perhaps because of an irregular and folded membrane around the heart fibers, giving a high membrane surface area. In contrast to resistance, capacity of the membrane undergoes no appreciable changes during excitation [36]. It may be assumed that capacity is simply an expression of the structural component of the membrane.

Conduction velocity: (θ) The velocity of a conducted impulse depends on these constants. Katz [59] has shown that for nerve tissue the square of the velocity is proportional to the fiber diameter and expressed it in these terms:

$$\theta \cong \frac{\sqrt{a}}{C_m \sqrt{R_i}} \qquad (7)$$

with symbols as before. Calculated velocities in normal tissues agree reasonably well with experimental observations based on measurements made with surface electrodes spaced at known distances from each other. The differences in conduction velocity of cardiac tissues, very high in Purkinje fibers, slower in ventricular muscle, very slow in nodal tissues (Table III), depend largely on the fiber diameter. Clearly, however, the conduction velocity in heart muscle must depend on additional factors, notably on the rate of rise of the action potential. This, for example, is faster in Purkinje fibers than elsewhere. (Table III.) Depolarization is a function of ionic conductance (*vide infra*), and the influence of various procedures, therapeutic or toxic influences on the conduction velocity in heart muscle may depend on these factors to an equal or greater degree than on those expected from the parameters listed by Katz.

IONIC BASIS OF CARDIAC EXCITATION. *Membrane potential and membrane current:* The time course of a transmembrane voltage (E), as recorded by intracellular electrode has been described. E denotes total voltage changes across the boundary. It is equal to the sum of the potential changes occurring within the membrane itself (E_m) and those occurring at the inner and outer interface (E_i and E_o). These components of E may be influenced by existing static charges at the boundary, inductance, and fixed charges within the boundary. E is related to differences in electrolyte concentration between the cell interior and the extracellular space. The electrical potentials to be expected by selective diffusion of certain ions across a

liquid boundary follow the Donnan equilibrium concept invoked for biologic tissues by Boyle and Conway [*19,30a*]. It assumes a membrane permeable to K^+ and Cl^- but relatively impermeate to Na^+ and Ca^{++}. The *permeability* of an ion (P_i) and its *conductance* (G) determine membrane potential (voltage: E_m) and membrane flux (current: I_m). Rigorous mathematical treatments of this dependence of diffusion potentials on the mobility of an ion and on differences in concentrations of electrolytes on either side of a semipermeable barrier have long been available in terms of Henderson's equation (for E_m only) or of the well known Nernst-Planck equation (for E proper) [*13,58,69*]. These state that the observed potentials are directly proportional to the logarithm of the rates of the concentration differences of an ion. Precise measurement of the dissociated ions in the intracellular space is difficult, but from the available data for heart muscle [*34,83b*] an *equilibrium potential* can be derived for each ion, defined as the potential difference at a ratio between intra- and extracellular concentrations where no net current will flow. (Fig. 2 and Table II.) For the three major ions involved this would be written

$$E_K = \frac{RT}{F} \ln \frac{[K]o}{[K]i}; \quad E_{Na} = \frac{RT}{F} \ln \frac{[Na]o}{[Na]i}; \quad E_{Cl} = \frac{RT}{F} \frac{[Cl]i}{[Cl]o} \qquad (8)$$

where R represents the gas constant, T the absolute temperature, F the Faraday constant, and the symbols in brackets the concentrations of ions, o on the outside, i on the inside. The equilibrium potential for K^+ is calculated for nerve, skeletal, and heart muscle at about minus 100 mv., for Cl^- for somewhat less, for Na^+ about plus 60 mv. Since the resting potential of all excitable tissues examined is close to E_K, free movement of this ion through the membrane is considered the major factor in maintaining a resting potential: P_K, the potassium permeability of the membrane, is about twice that for Cl^-, and twenty-five times that for Na^+. Slight changes in [K]o alone should therefore change transmembrane voltage E (providing such changes in [K]o will not promptly alter [K]i). Experimental observations agree with this concept which was expressed earlier by Bernstein [*11*]. Precise measurements bearing on this relationship are difficult to obtain; the equations would not hold, for instance, if the membrane were not neutral but were to contain fixed charges making it acid, basic or amphoteric in character. Changes at the inner and outer boundary of the membrane by induction or otherwise could alter this relationship in either direction. Attempts to extend the Planck equation to fit these modifying concepts have been made [*58,94*].

A resting membrane potential approaches but does not equal values calculated for E_K (Table II), and increasing the gradient of K^+ across the membrane by lowering or removing [K]o reveals deviations from the expected value [*8,53,94*]. In Purkinje fibers bathed in K^+ free tyrode solution, E falls toward a new stable value which is low (approaches the zero line) although under the circumstances it would be expected that E should increase and eventually equal E_K. (Fig. 3.) This anomalous behavior may involve selective layering of K^+ on the outside boundary and need not invalidate the general case. If one assumes an influence of other ions on the resting potential [*53*] in order to explain why E is not E_K, an expression can be given

$$E = \frac{RT}{F} \ln \frac{[K]o + \alpha[X]o}{[K]i + \alpha[X]i} \qquad (9)$$

where X represents an ion with equilibrium potential opposite to that for K^+. The presence of this ion will decrease E, and move the membrane potential farther away from the level of E_K. α represents a constant expressing the ratio of permeabilities of the two species of ions considered. If X were Na^+, the presence of a small, steady inward flux of Na^+ in the resting state would explain why E does not approach E_K. α would then represent the apparent permeability of Na^+ relative to that of K^+. Figure 6 shows a slight increase in E for Purkinje fibers when Na^+ is removed from the extracellular compartment. A much larger resting Na^+ inward current seems to be characteristic of pacemaker tissues [*98,99*] where apparently for this reason a low membrane resting potential is found. (Table I.)

Of late, the permeability to chloride ions and its contribution to the resting potential has been considered [*15,24,53,55*]. For skeletal muscle, chloride conductance contributes significantly to the resting total membrane conductance. When in these experiments Cl was replaced by SO_4, changes were noted "due to SO_4" which depended, however, on binding of Ca^{++} by sulfate. This, of course, causes effects similar to

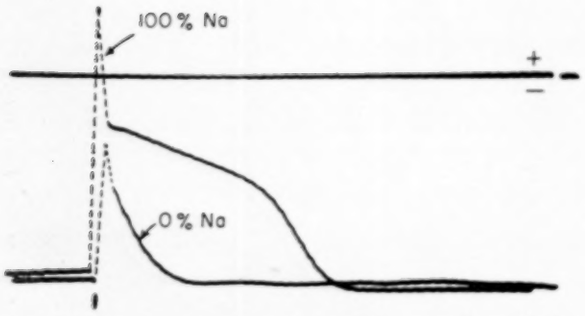


FIG. 6. Action potential of Purkinje fiber of sheep before and after replacement of extracellular Na^+ by choline chloride (Weidmann and Hecht, unpublished). Note slight increase in membrane potential, disappearance of overshoot, failure of complete depolarization and shortening of the duration of the action potential on sodium depletion.

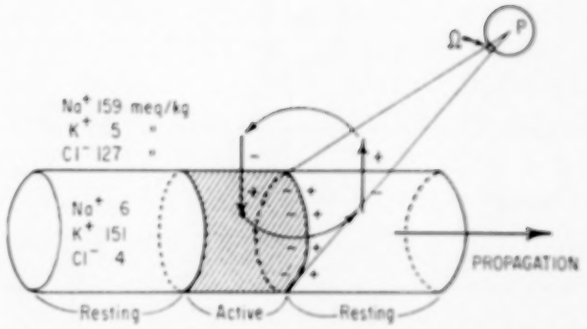


FIG. 7. The local concept of excitation (see text). P is a point from which a surface record may be obtained. Ω is the solid angle, defined as the area cut out upon a spherical surface of unit radius inscribed about P as a center by a certain cone. This cone is formed by the lines from P to the boundary of acting tissue. An observer looking through this cone will at this instant see the positive side of the membrane surface, Ω will be positive. Data for intra- and extracellular electrolyte concentration are given in m Eq./kg. and vary for different tissues and different fiber types [*31,83a*].

those seen in Ca^{++} free solution (*vide infra*). In heart muscle, Cl^- does not influence the diastolic resting potential but seems to be involved in carrying electric charges during depolarization [*24*].

On *excitation*, current flows along the axoplasm from activated to resting area, through the membrane, over the fiber surface from resting to active area, and through the membrane again, in a local circuit. (Fig. 7.) The internal flow through the axoplasm triggers contractile mechanisms, and will not be of further concern. The external current flow will be discussed in relation to the form of the electrocardiogram and volume conduction. The membrane currents depend on changes in ionic permeability and conductance. Excitation, in general, and initiation and propagation of the heart beat, in particular, are the consequences of turning on and off these ionic membrane currents by changing the resistance of the membrane (or increasing its conductance) to the passage of ions.

Such current flow through the membrane can be expressed by the cable equations mentioned where the current is given in relation to the propagation of the action potential along the fiber [*14,27*]. It is stated in terms of a second partial derivative of the membrane potential:

$$I_m = \frac{i}{r_0 + r_i} \cdot \frac{\delta^2 E}{\delta x^2} \qquad (10)$$

where x is distance along the fiber, I_m the membrane current per unit length, the other symbols as before. In this form it is not a useful equation. If changed to an ordinary second derivative of the membrane potential [*51*]

$$I_m = \frac{a}{2R_M\theta^2} \cdot \frac{d^2V}{dt^2} \qquad (11)$$

it remains complicated and in addition contains an unknown, θ, the velocity of conduction (see equation 7). If one omits any consideration of conduction due to local circuits of the kind discussed, the membrane current can be divided into two components, the ionic current I_i, and the current associated with the condenser properties of the membrane, the capacity current. Membrane current density (in amp./cm.^{2}), I_m, can then be expressed as follows:

$$I_m = \underset{\text{ionic current}}{I_i} + \underset{\text{capacity current}}{C_m \frac{dv}{dt}} \qquad (12)$$

Since on excitation both voltage and resistance change, I_m cannot be obtained readily although the biologic membranes under consideration obey Ohm's law. To avoid these complexities a preparation was developed which denied all impulse propagation to the cell by preventing longitudinal current flow. This leaves the membrane potential and membrane current flow the same over the length of the fiber, using electronic feed-back guard electrodes on either end of the fiber [*70*]. In addition to this "space clamp" a second feed-back system was installed which fixes membrane potentials at arbitrarily selected

values. This "voltage clamp" prevents spontaneous excitation and minimizes capacity currents. With this complex experimental preparation Cole [*25,26*] and Hodgkin [*49,51,52*] were able to obtain the desired information concerning I_L. Hodgkin and Huxley, in particular, have painstakingly delineated the nature of ionic currents, at least for isolated nerve fibers. From these studies it appears that excitation is associated with rapid inward flow of Na^+ followed by a sustained outward flow of K^+. Obviously, this requires changes in permeability constants occurring on excitation which are best expressed in terms of ionic conductances (in mmho/cm².). Since membrane conductance is the inverse of resistance of the membrane to the passage of an ion the following relation holds:

$$G = \frac{I}{E} \tag{13}$$

and for individual ions:

$$G_{Na} = \frac{I_{Na}}{E - E_{Na}}; \quad G_K = \frac{I_K}{E - E_K}; \quad G_L = \frac{I_L}{E - E_L} \tag{14}$$

where G_L represents conductance to other ions such as Cl(I_L "leakage current" of Hodgkin and Huxley). The total ionic current is the sum of its parts: $I_m = I_{Na} + I_K + I_L$. By rearranging and substituting into equation 11, a simple equation can be given:

$$I_m = C_m \frac{dV}{dt} + G_{Na}(E - E_{Na}) + G_K(E - E_K) + G_L(E - E_L) \tag{15}$$

Hodgkin and Huxley went further, and developed a set of equations on an empirical basis in an attempt to describe in greater detail the permeabilities as related to time and to the membrane potential. They express

$$G_K = \bar{g}_K n^4 \tag{16}$$

where $\bar{g}_K$ is a constant and n a dimensionless variable, the proportion of particles in a certain position on or within the membrane. Change of n in time is a function of the membrane potential E. According to this, K^+ can traverse the membrane only when four charged particles are located at a certain area of the membrane under

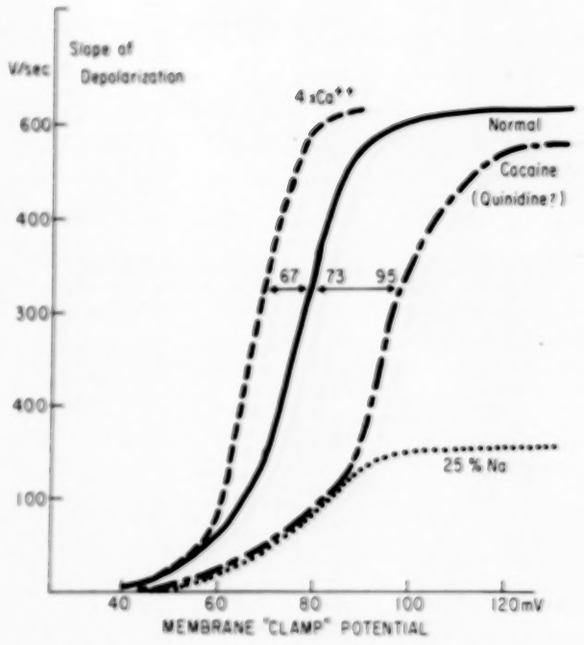


FIG. 8. Relation of membrane potential and depolarization slope (sodium carrier system). In normal fibers the slope of depolarization (a measure of sodium inward current I_{Na}) depends on membrane resting potential, with a maximum value reached at 90 mv. This is a function of the mechanism which carries Na^+ through the membrane on excitation. The curve is shifted to the right (blocking the carrier system) by surface anesthetics, quinidine, and when external sodium concentration is decreased. It is shifted to the left in solution with high external Ca^{++} (Weidmann [*9,103,120*]). A slope value observed in normal tissue at 73 mv. will already be reached at 67 mv. in Ca^{++} rich medium, and only at 95 mv. under the influence of cocaine. Slope values are approximate and vary with different hearts and different fiber types. (Table III.)

the influence of the electric field. Similarly,

$$G_{Na} = m^3 h \bar{g}_{Na} \tag{17}$$

where Na conductance depends on three events of probability, m, which allow Na^+ to pass and one event (1 − h) to block Na^+ transfer; m^3h means that there are three activating particles and one non-blocking particle. Collecting these terms, a workable expression for membrane current density for 1 cm². of membrane can be given:

$$I_m = C_m \frac{dV}{dt} + \bar{g}_K n^4 (E - E_K) + \bar{g}_{Na} m^3 h (E - E_{Na}) + \bar{g}_L (E - E_L) \tag{18}$$

E and E_K may be measured directly; the ionic conductances are given by the constants $\bar{g}$, and

the quantities n, m, and h, which change with time and with the membrane potential, may be obtained from subsidiary equations developed by Hodgkin and Huxley [*51*].

The outlines of these mathematical steps are given because the voltage clamp experiments have become essential to any understanding of the excitatory process. The somewhat empirical formulations have made it possible to examine various components of membrane behavior, and to reconstruct quite precisely action currents of various fibers (*vide infra*). Although designed to fit only the excitatory events in nervous tissue, they are vital to further progress in related fields such as the study of cardiac excitation and recovery.

Ionic flux in cardiac membranes: The dependence or interrelation of the cardiac resting potential on the ratio of potassium concentration on either side of the boundary has already been mentioned. G_K is high but due to the existing potential difference K^+ is held within the fiber and therefore I_K is low. Total membrane resistance, R_m, is low at the beginning of diastole but increases steadily until depolarization takes place. (Fig. 2.)

Depolarization: The general case for the ionic basis of excitation also holds for heart muscle: at the instant of depolarization a sharp decrease in R_m occurs. (Fig. 2 and Table III.) Depolarization and overshoot depend on an increase in membrane permeability which allows Na^+ to enter the fiber rapidly. The speed of this reaction depends on (1) the level of resting potential preceding excitation (Fig. 3, Table III) and (2) the concentration of Na^+ in the extracellular space: $[Na]_o$. The upstroke velocity of the depolarization spike (mv./second) and the size of the overshoot are therefore a measure of the sodium inward current: I_{Na}.

Transport of Na^+ through the membrane requires a carrier whose activity is at a maximum above 90 mv. resting potential [*103*]. (Fig. 8.) Interference with the carrier system causes slow depolarization and loss of overshoot, and eventually loss of excitability in the face of a high resting potential; the cell is considered "stabilized" and polarization is "locked." Such effects are seen following the administration of quinidine, procaine amide, and other antiarrhythmic and antifibrillatory compounds. It appears that they exert their action on heart muscle by virtue of their influence on the mechanism which carries Na^+ through the membrane [*103*]. (Fig. 8.) The phenomenon of threshold, the "firing level," and the slow diastolic depolarization of pacemaker regions is likely to be a function of this system as well. Threshold voltage may be defined as the potential at which sodium inward current exceeds the modest outward current (K^+ out, Cl^- in). The level of threshold, therefore, depends on the ratio of I_{Na}: $I_K + I_{Cl}$. Less sodium inward current and less potassium outward current will raise threshold for excitation, an increase of sodium inward current or interference with the Na^+ extrusion mechanisms (*vide infra*) accomplishes the opposite and will make a cell more excitable.

Ca^{++} ions are also considered membrane stabilizers. This ion has no direct effect on the magnitude of resting and action potential but shifts the firing threshold toward lower values, thus reducing cellular excitability, i.e., it will take a greater amount of depolarizing cathodal current to induce the all or none effect of spontaneous firing. Ca^{++} enhances the activity of the carrier system for a given membrane potential (Fig. 8), but it has no effect on the maximum transport capacity, in contrast to the pharmacologic stabilizers mentioned. Following a decrease in $[Ca]_o$ the firing threshold moves toward E_K. Excitability is therefore increased since firing can now take place at a higher resting potential. In Purkinje fibers a reduction in extracellular Ca^{++} to 25 per cent of normal invariably induced spontaneous activity in a previously quiescent preparation. There is evidence for cardiac tissue that Ca^{++} competes with Na^+ at the membrane surface, and that, by reducing $[Ca]_o$, Na^+ inward current is enhanced and excitability increased on this account [*8,39b,67*]. There is some doubt that Ca^{++} depletion can induce spontaneous activity in ventricular fibers [*99*]. The statement that all cardiac tissue possesses the capacity for spontaneous impulse formation must be re-examined in the light of these observations.

Repolarization: Restoring the resting equilibrium requires a more complicated interplay of electrolyte transports. In nerve, a brief sodium inward current at the instant of depolarization is followed by a prolonged potassium outward flow [*4,51*]. In heart muscle, detailed studies on the recovery mechanism are sparse. The long plateau, however, which is so characteristic of cardiac fibers, points to certain peculiarities in the time course of ionic currents of cardiac

membranes which are not generally shared by other tissues. It is assumed that sodium conductance, G_{Na}, decreases rapidly at the height and following the overshoot (as in nerve tissue), and that it remains well above diastolic resting G_{Na} during the plateau. G_K, high at all times, increases further during this phase and reaches a maximum during the final stage (phase 3) of repolarization. The accumulation of K^+ on the external surface of the membrane due to the high potassium permeability which must occur at the end of phase 2 (the plateau) might be directly concerned with the return of membrane potential to the resting level. The plateau then might be considered a state of the membrane where G_{Na} and G_K are in balance: a decrease in G_{Na} or an increase in G_K or opposite changes in both should restore the resting potential E prematurely and thereby shorten the duration of the action potential. The ease with which plateau and action potential duration can in fact be altered is therefore not surprising since interference with several normal mechanisms can be expected to alter the G_{Na}/G_K ratio.

Shortening of the plateau can be expected and is observed by (1) decreasing external Na^+ (decreasing I_{Na} inward current) (Fig. 6) and (2) increasing external K^+ (decreasing I_K outward current) [*18*]. Metabolic inhibitors, anoxia, and digitalis glycosides shorten the action potential presumably by increasing internal Na^+ due to interference with the Na^+ extruding mechanisms, while the shortening induced by acetylcholine depends for its effect on G_K (*vide infra*) [*8,98*]. Lengthening of the plateau is seen by (1) decreasing external K^+ and (2) increasing external Na^+. Veratrine, Ca^{++}, quaternary ammonium compounds and quinidine lengthen the plateau, possibly by interfering with the normal decline of G_{Na}, barium and desoxyglucose (in frog muscle) by retarding the expected increase in G_K. It has been suggested that the ionic interplay responsible for the prolonged action potential of cardiac muscle may be energy-dependent and that alterations in K^+ may *per se* influence oxidative phosphorylation and the release of ATP [*68*].

Much has been said about an active transport system (a "pump") which maintains a Na^+ differential across the membrane. Since sodium inward and potassium outward movement occur in the direction of the chemical gradient, no energy is required for this "passive" membrane transport. Although the amounts transferred are minute, an active cell would gain Na^+ and lose K^+ with each beat unless an extruding mechanism for Na^+ and a loading mechanism for K^+ are assumed in order to restore the resting state. Such ionic movements would have to work "uphill" against an electrochemical gradient. "Active" transport which consumes energy must take place. This pumping mechanism is poisoned by inhibitors such as dinitrophenol, cyanide and iodoacetic acid and must therefore be metabolically driven [*52*]. Experiments on non-medullated nerve fibers suggest a coupling mechanism: for each quantity of Na^+ extruded a similar quantity of K^+ is syphoned into the axoplasm. As one might expect, the energy required for active transport is provided by ATP or some other energy-rich phosphate compound [*22*].

There is evidence that in spontaneously beating muscle fibers (pacemaker tissue) an increased inward leakage of sodium exists. Apparently, this increased G_{Na} causes the slow diastolic depolarization so characteristic of pacemaker tissues when potassium conductance (G_K) is reduced during diastole. The fact that the membrane resting potential (E) of the sinus region is less than elsewhere (Table 1), and that membrane resistance increases in this area prior to excitation supports the concept of a high "steady state" sodium conductance and a reduced G_K during diastole as the basis for automaticity. Cathodal stimulation above threshold or low external Ca^{++} concentrations can induce pacemaker activity in previously quiescent fibers by virtue of an increase in G_{Na} [*99*]. The demonstration that sodium carrier activity is already fully active at the end of repolarization [*103*] is not necessarily against this. The well known inhibitory effect of acetylcholine on pacemakers on the other hand has been clearly demonstrated to depend on changes in G_K only [*8*]. The action of epinephrine, the occurrence of spontaneous activity in previously quiescent fibers, onset of oscillations, and repetitive firing are apparently independent of K.

Subsidiary evidence for the ionic basis of cardiac excitation: The experimental observations leading to present concepts of the nature of excitation have been subject to verification by two independent methods: ionic fluxes and net entry or outflow of ions can be studied with radioactive tracers, and the mathematical analyses derived from experiments can be programmed for a variety of conditions on a com-

puter and the resulting curve checked against the experimental tracings available.

Measurements of membrane transfer of Na^{24} and K^{42} during nervous activity were made by Keynes [*62*] and by Keynes and Lewis [*63*]. There is good agreement between the quantity of Na^+ which enters the cell during one impulse and the amount of K^+ which leaves it. It appears that the amount exchanged is higher for skeletal muscle than for nerve. For heart muscle, radioactive tracer studies (less numerous and less conclusive) reveal flux data which are higher than those reported for skeletal muscle [*30b*,*57*, *112*]. Wilde [*105*] has demonstrated that release of K^{42} from turtle ventricle is pulsatile and that peak outflow of K^+ occurs toward the end of the plateau and during the rapid final descent (phase 3) of the repolarization process. This observation would fit well with the phasic fluctuation in G_K assumed for the cardiac cycle. Release of K^{42} was seen to depend linearly on heart rate, and to increase during the effect of actycholine, again a predictable result [*112*].

The minimum transfer of charges across the membrane is determined by the action potential and the membrane capacity as follows [*4*]:

$$MT = \frac{C_m \cdot V}{F} \qquad (19)$$

where MT is a minimum transfer (in coulombs), V the total action potential, C_m membrane capacity, and F the Faraday constant. On this basis a charge held on the resting membrane of ventricular fiber of frog would be 12 μF $\times$ 80 mv. = 960 mμ coulombs, with the reversed charge on the active membrane: 12 μF $\times$ 15 mv. = 180 mμ coulombs. The quantity of charges transferred on excitation would equal 1.140 μ coulombs. Based on such calculations, a minimum of from 10 to 12 $\mu\mu$ M/Na^+/cm.^{2}/beat would be required to enter the fiber for spontaneous depolarization. (Table IV.) Based on the kinetics of uptake and release of Na^{24} with sucrose as the extracellular space indicator, Johnson calculated a net Na^+ entry of 15 $\mu\mu$ M/cm.^{2}/beat [*57*]. This is in good agreement with the estimates made from the electrical constants and leaves a reasonable "safety factor." In addition, flux data such as these are a powerful support for the sodium hypothesis of excitation. For heart muscle the energy required by the pump to remove these amounts from the cell by active transport and to move K^+ back into the cell (if one-to-one coupling exists) is given as 8×10^{-2} cal./gm., or 13 per cent of the total energy liberated [*57*].

The second approach toward verification of the concepts consists in computing action potentials under a variety of conditions from the Hodgkin-Huxley equations (15, 16, 17 and 18). From these, Hodgkin calculated nerve action potentials in 1952 [*51*]. These data have been extended by Cole [*28*] and by Huxley [*56*]. By changing the appropriate constants, the formula yielded computed action potentials for repetitive discharges, oscillations, and changes in threshold closely resembling recorded curves. By decreasing values for G_K and increasing the time constants for the changes in conductance, the Hodgkin-Huxley equation (18) gave calculated action potentials with a prolonged plateau identical with those obtained from cardiac muscle or from medullated nerve fibers treated with TEA [*38*]. Figure 9 illustrates a computed cardiac action potential and the presumed time course for changes in ionic conductance at an E_K of minus 100 mv., and E_{Na} of plus 40 mv. [*80*]. Changing m of equations 17 and 18 (the factor for sodium conductance) and increasing G_K yielded pacemaker potentials with the characteristic slow diastolic depolarization, demonstrating the sensitivity of pacemaker potentials to changes in ionic conductance, a situation similar to that observed in experiments. The computer studies of FitzHugh [*38*] and of Noble [*80*] clearly demonstrates that the equations of the Cambridge group will give the desired solution not only for nerve and skeletal muscle but for heart muscle as well. This does not imply, however, that a different set of equations may not be equally applicable.

BASIC ANALYSIS OF A SURFACE ELECTROCARDIOGRAM. An extracellular record of cardiac excitation (electrocardiogram) depends on the time course of the transmembrane currents, the possible differences of these events between cardiac fibers, and on the properties of the volume conductor surrounding the fiber.

The surface action potential: At the height of excitation, during the maximum rise of Na^+ inward current, a rapid deflection (QRS) is seen in surface records, a near isoelectric period (R-ST segment) during the plateau, and a slower deflection (T) during the final phase of

the return to the diastolic resting potential. (Fig. 2 and 10.) Clearly, only rate of changes, $\frac{dV}{dt}$, is seen at the surface, while the magnitude of sustained potential differences, and presence and size of overshoot are not represented. It is also obvious that changes in the duration of excitation will be reflected in a lengthening of the surface records. Furthermore, changes which cause a delay in the time course of depolarization should cause widening of QRS, and changes in the rate of return to the diastolic membrane potential should influence the configuration of T. In neither case need one evoke as an explanation of the changes in QRS and T the interplay of cells and the differences in electrical behavior between them (i.e., the ventricular gradient). This applies at least in part to the alterations of QRS, "QT," and T observed during the action of quinidine, procaine amide, external electrolyte changes (notably $[K]_o$), digitalis, anoxia and many others.

A concept of the nature of the process of propagation of an action potential is given in Figure 7. This has been referred to as the "local circuit theory." Once a region is excited or undergoes spontaneous activation, a cathodal (depolarizing) current flows in the axoplasm in the same direction as the "impulse" travels over the fiber. The axoplasmic current draws charges from the membrane potential there until threshold is reached. At that instant, R_m declines, Na^+ permeability sharply rises, and the fiber is depolarized. In the external medium a current flows in the opposite direction, from the resting to the active site. A point on the surface of the fiber just prior to and ahead of the beginning electrotonic removal of capacity charges is con-

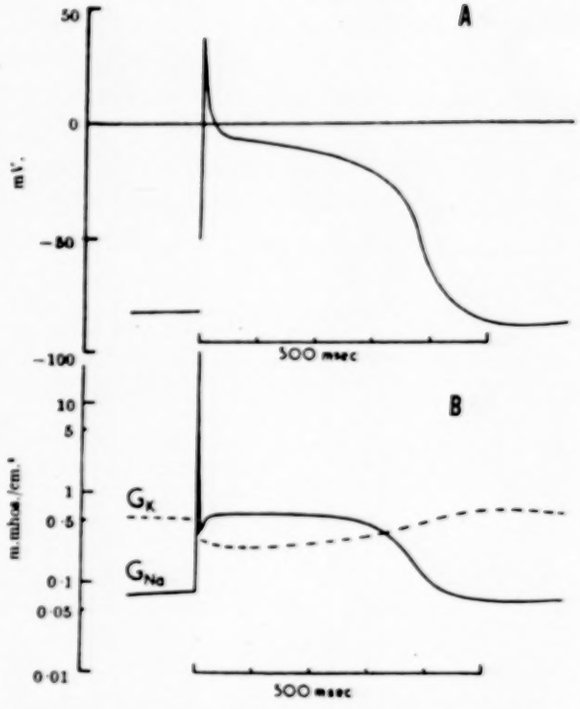


FIG. 9. A, a computed action potential of cardiac muscle on the basis of the Hodgkin-Huxley equations. B, computed changes in membrane conductance for cardiac muscle demonstrating the interplay of G_K and G_{Na} during the plateau where G_{Na} is about 8 times the resting value, and G_K is lower than during diastole. During onset of excitation G_{Na} spikes to a very high value (see text) (from Noble [80]).

TABLE IV

IONIC FLUX ASSOCIATED WITH EXCITATION*

Fiber	Species	Diameter (μ)	C_m ($\mu F/cm^2$.)	K^+ out ($\mu\mu$ M/cm².impulse)	Na^+ in ($\mu\mu$ M/cm².impulse)	MT
Heart, ventricle	Frog	10	(12)†	20	15	10
Heart, ventricle	Dog‡	16	10	5.5– 9.0† 7.3–12.0	11–18‡	
Skeletal muscle	Frog	135	8	9.3	14.9	6
Skeletal muscle	Rat	100	5	16.0	19.0	6
Unmyelinated nerve	Lobster	200	1	4.3	3.7	1.6
Unmyelinated nerve	Squid	500	1.5	3.0	3.5	1.6

NOTE: C_M = membrane capacity; MT = minimum ionic transfer required for excitation (see text).

* [4,7,30b,30c,52,57,62,63,112].

† C_M for kid Purkinje fiber.

‡ Data by courtesy of H. L. Conn. First figures are given with, second figures without 1.6 correction factor for fiber shape. For K^+ upper figure indicates perfusion experiment, lower figure intact animal.

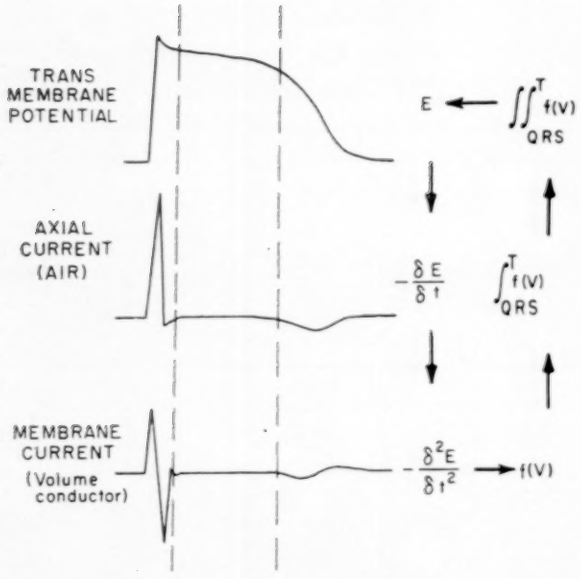


FIG. 10. The relation of membrane potential to surface records (see text).

sidered a "source"; the height of G_{Na}, the time of maximum sodium inward current, a "sink." Both source and sink are the result of current flowing in a local circuit and are the expression of changes in membrane conductances. They cannot therefore be considered separately and are treated as a singularity termed a "dipole" or electrical doublet. The notion of the dipole as a vector quantity with limited position and magnitude is clearly contained in Helmholtz's treatment of the principles of electromotive surfaces [*12*], and was an important implicit assumption made by Einthoven in his triangle analysis [*37*]. Using the Hodgkin-Huxley concept, "propagation," "impulse," "action current" in the accepted sense of electrocardiography are then an expression of a wave of increased sodium permeability spreading along the fiber and from one fiber to the next. For the relatively simple structure of nerve, a mathematical treatment of the action current in a volume conductor has been given by Lorente de Nò [*79*], and a general treatment for currents generated by excitable tissue submerged in a volume conductor is available in the classic monograph by Wilson [*10*]. Since these are special cases of potential functions used in circuit analysis which describe the flow of currents in volume conductors [*64*] only a brief summary will be given here.

The potential differences measured between two points on the surface of a *suspended* fiber measure the longitudinal current along the fiber; it may also be considered to measure the rate of changes in net charges at the membrane with time [*86*]. By integration such a curve will give a monophasic action potential, the transmembrane voltage, the integration constant being the resting membrane potential. A surface record obtained *in situ* is different and may be represented by the rate of change of the measured potential differences. It is therefore identified with membrane current density, or the second derivative of the transmembrane potential:

$$V = \frac{\delta^2 E}{\delta x^2} = I_m \cdot r_e \qquad (20)$$

where V is the potential measured, E the membrane potential, I_m and r_e the density of the transmembrane current and the external resistance respectively. Figure 10 indicates this relation for cardiac muscle. It is seen that one form of recording can be transformed into another by integration (ECG to MAP) or differentiation (MAP to ECG), and that a surface action potential may be considered an approximate measure of the membrane action current, records from isolated excised tissue suspended in air a measure of the membrane potential. Graphic differentiation and integration can be performed and will allow reconstruction of normal and abnormal electrocardiograms from membrane potentials, and of transmembrane potentials from the electrocardiogram. Integration remains incomplete, however, i.e., a transmembrane potential cannot be reconstructed from a surface electrocardiogram because the integration constant (membrane resting potential) cannot be estimated from surface records. Nevertheless, a general relation is apparent in Figure 10.

Dipole moment and volume conduction: It is not useful to pursue this analysis further without reference to the position of the recording electrode in the volume conductor. If one electrode is placed at ground (infinity, "indifferent electrode") the potential variations obtained are predominantly those recorded by the electrode close to the excitable tissue. In a homogeneous sheet of infinite extent these are expressed by:

$$V = K'(e \cdot l) \log_e \frac{r_1}{r_2} \qquad (21)$$

where e represents the charge, or quantity of electricity flowing per unit time, l the distance

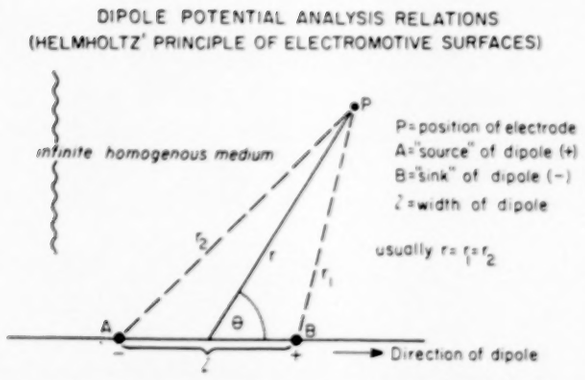


FIG. 11. The relation of a dipole (source B, sink A) to a point P located anywhere in a conducting medium. l is usually small compared to r_1, and r_2. This can also be expressed by the solid angle subtended at P (see Figure 7).

between sources and sink, r_1 and r_2 the distances of the electrode from source and sink, respectively [*10*].l is very small and $r_1 = r_2$ in most biological situations. $K^1 = \frac{1}{2\pi kd}$ a dielectric constant expressing conductivity (k) and thickness of the medium (d). Depending on the nature of the conducting material, equation 21 transforms to the general form for a homogeneous medium of infinite extent:

$$V = K''(e \cdot l)\frac{\cos\theta}{r^2} \qquad (22)$$

where θ is the angle made by the dipole axis (a line joining source and sink) with the point p, the position of the electrode. Figure 11 illustrates the factors which enter equation 22. K'' is given as:

$$K'' = \frac{1}{4\pi kd}$$

Canfield [*23*] has calculated the potential of a dipole in the center of a sphere

$$V = K''(e \cdot l)\cos\theta\left(\frac{1}{r^2} + \frac{2r}{R^3}\right) \qquad (23)$$

where R is the radius of the sphere. If R is large, equation 23 reduces to equation 22. Equation 23 has served as the first practical equation for field studies on cardiac muscle reported by Craib, the first author to formulate a doublet hypothesis for cardiac excitation [*32*]. Additional equations for a variety of shapes of volume conductors are given by Wilson [*10*]. From these, surface potentials could be calculated which showed a striking similarity to those obtained experimentally. Records from the human heart

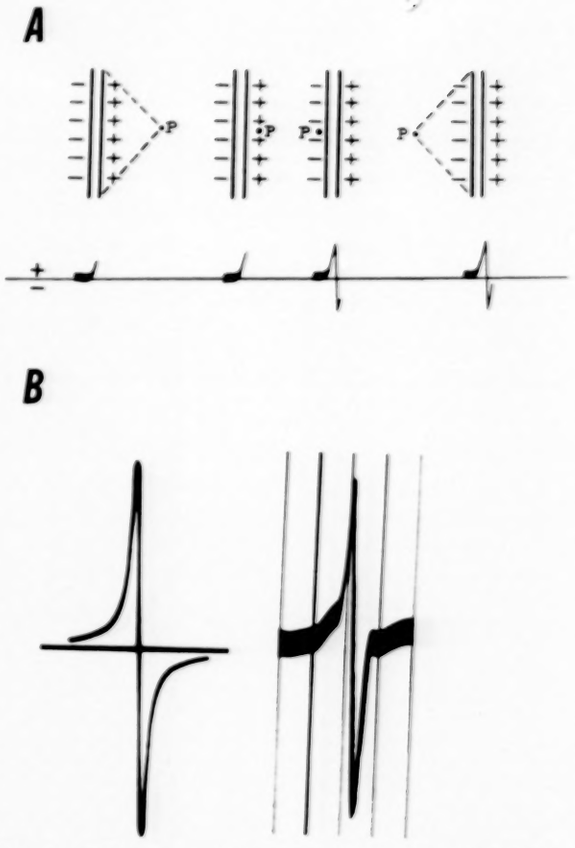


FIG. 12. A, the passage of a wave of excitation. P defines a point representing the location of the exploring electrode. As the dipole passes over P, the potential variations recorded will swing from a maximum positive value to a maximum negative value as indicated in the tracings. B, a calculated and an observed action potential, the latter obtained from the atrial surface of human heart, the former from equation 22. The configuration of these curves suggests that excitation may be expressed by a series of dipoles moving through a volume conductor as schematized in (A). (After Hecht and Woodbury [*45*].)

in situ are equally predictable from these equations [*45*]. These studies provide convincing evidence that the laws which govern distribution of electric currents in volume conductors are applicable to the analysis of a surface electrogram. Wilson [*10*] has restated these equations in three dimensional form substituting for θ and r the solid angle Ω subtended by the dipole layer at the point p of Figure 11, and expressing the first part of the equation 22 as φ. Equation 22 then can be written simply as:

$$V = \phi\Omega \qquad (22a)$$

Analyses using the solid angle are carried out in detail by Bayley [*1*]. (Fig. 7.)

Equations 21 to 23 are based on Helmholtz's

treatment of the Laplace equation which states that the sum of all potential measurements in an infinite medium is zero. The field equations derive from this law, for equation 23 for example no current will flow across a circular lamina if r = R.

Several additional statements emerge from the use of these equations:

(1) The potential of a given point is proportional to the quantity of current flowing from source to sink given as (e · l) or m′, the proper unit of dipole strength or dipole moment (charge times distance).

(2) All evidence clearly implies that excitation of tissue submerged in a volume conductor is represented by a source-sink arrangement, and that such curves signal excitation by a positive deflection as the source passes the electrode, and that this is immediately followed by a negative deflection as the sink arrives.

(3) The passage of the excitation wave under the electrode when $\theta = 90°$ is indicated by the mid point of the plus minus deflection so obtained. (Fig. 12.) The distance between maximum positivity and maximum negativity in a lead recorded from the cardiac surfaces was termed the "intrinsic deflection" by Lewis [*65b*]. It is a valid measure, irrespective as to whether or not it can be equated with the downstroke of a QRS complex in a precordial lead. It is not a precise measure, however, when obtained by the use of a unipolar exploring electrode: Small contiguous bipolar electrodes placed a minimal distance apart on the activated tissue give a more correct indication of the onset of local depolarization [*85*].

(4) The magnitude of the potential variations so recorded varies inversely with the distance of the electrode from the generator source. The essential relation of V to $1/r^2$ (a Newtonian potential) has not been fully appreciated by those who consider a record obtained from any area of the surface of the body equidistant from the generator, and therefore of no particular "localizing" value, or by those who claim that precordial or direct surface leads represent exclusively the potential variations of adjacent areas. Wilson expressed this clearly in 1930 [*106*]: "The potential variations of the electrode which is placed close to the heart will not only be very much greater than those of the distant electrode, but they will represent the activities of the various portions of the heart *unequally*. Those portions of the heart which are nearest to the electrode which is nearby must exert a very much greater effect in proportion to the potential differences which they produce than those parts of the heart which are further away . . . It is obvious, however, that an electrode which is placed upon the heart bears no special relation to the subjacent muscle except that of nearness . . . " The relation is not a simple one since the localizing value of a precordial lead (the ability to record changes from adjacent areas preferentially) depends on the distance from the location of the dipole, which changes from instant to instant. Not all precordial leads are semi-direct (i.e., resemble a direct lead), nor do all parts of a precordial curve have localizing characteristics (i.e., are predominantly influenced by adjacent areas during the inscription of the curve). The simple consideration given in Figure 13, where heart and chest are drawn to scale, illustrates the fact that for an electrode position at B and C (left lateral chest and back) the three dipoles D-x, D-y, and D-z, located at various points within the heart, can be considered equidistant. For an electrode in location A (anterior chest) a dipole at D-x has a six times greater influence than one located at D-z. Experiments carried out by Hartmann et al., using hearts suspended in a conducting medium have indicated that considerations of this kind become crucial when the distance of the electrode from the heart reduces to less than twice the cardiac diameter [*42*]. In model experiments designed to examine the relationship of the cardiac generator to the surface lead the heart is usually represented as a single "equivalent" dipole, a notion that has its origin in Einthoven's basic paper [*37*]. In such experiments $V = 1/r^2$

TABLE V
DIPOLE MOMENT CALCULATIONS*
(TWO-DIMENSIONAL)

Species	Heart Size (gm.)	M′ (μAmp. cm.)
Leopard frog (R. pipiens)	0.14	1.08
Bull frog (R. catesbiana)	1.21	45.03
Man†	300.	1000.00

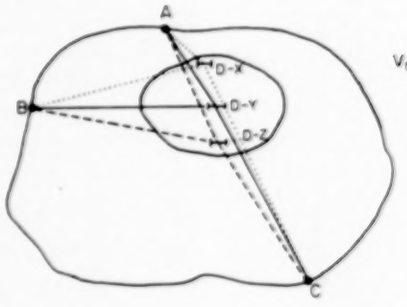
* Nelson and Hecht (unpublished, see [*75*]).
† Calculated from Gabor and Nelson [*40*]:

$$M' = \frac{V}{K'} \times \frac{r^2}{\cos \theta}$$

is occasionally neglected when it is argued that all parts of the heart give equal representation at any point on the surface. To the extent that the assumption of the heart as a single dipole may not be valid under all circumstances the conclusions of the model studies are limited in their practical applications.

Little thought has been given to the actual value of the external current flow between source and sink, the *dipole moment*. By a method whereby two measurements are made at right angles, θ can be calculated if E is known. Equation 22 can then be solved for m′, at least for a two-dimensional medium [*75*]. Table v and Figure 14 demonstrate that the sum total of cardiac current density for each instant of time is a vector which varies widely with heart size, or the number of fiber units involved in excitation. The time course of cardiac excitation of a bullfrog of Figure 14 is a vector which represents the true measure of a resultant (summed) change in membrane permeability passing from fiber to fiber. To this function the clinical vectocardiogram is closely related.

Differences in the duration of the excitatory state: The process of cardiac excitation as a whole is viewed as a composite resultant of its many individual units (fibers). Problems arise which are concerned with essential differences in the time course of membrane conductance changes of cardiac fibers activated simultaneously or in close time relationship to each other. These are expressed primarily in differences in the length of the plateau of one fiber as compared to another. Such differences in the duration of the excitatory state are the basis of the "ventricular gradient" of Wilson [*107*]. This concept is called upon to explain alterations in the recovery deflection, T, which are not the consequence of changes in the order by which an action current passes over cardiac musculature (changes in QRS). The calculation of the gradient is based on vector analysis similar to that underlying Einthoven's treatment of one-dimensional quantities (the "electrical axes") projected on the frontal plane but is based on two dimensional measurements of the area under QRS and T. The area of QRS-T, which is the ventricular gradient, has no physical meaning beyond the statement that it expresses differences in the duration of the excitation process of one region compared to another, and that a vector representing this value points from regions where systole is longer to where it is shorter [*48*]. The



$$\text{Dipoles } x,y,z \qquad V_{magn.} = \frac{M' \cos\theta}{4\pi K r^2} = \frac{K}{r^2}$$

	A		B		C	
	cm	$\frac{C}{r^2}$	cm	$\frac{C}{r^2}$	cm	$\frac{C}{r^2}$
X	4.3	.054	14.5	0.005	20.0	0.003
Y	8.0	.016	15.0	0.004	16.0	0.004
Z	10.5	.009	15.5	0.004	13.5	0.005

$$C = \frac{M' \cos\Theta}{2\pi K}$$

FIG. 13. A cross section of heart and thorax at Th 9. Imaginary dipoles located at D-x, D-y and D-z within the heart. A, B and C are locations for an exploring electrode. The magnitude of potential V at A, B or C produced by D-x, D-y or D-z is given by equation 22. The table gives actual distance of each dipole from the exploring electrode. The figure C expresses in arbitrary units V expected values at points A, B and C due to x, y and z. At A, V due to x would be six times larger than V due to z (0.054 vs. 0.009), whereas at B and C the magnitude of V is equal for all three dipoles.

presence of this vector quantity suggests that in heart muscle certain forces act upon different parts with different intensities. It is apparent that in the normal subject these factors prolong the excitatory state at subendocardial regions and shorten it at subepicardial regions [*43,44,65a*]. It is not clear what these forces are: temperature differences, stretch, tapering of the fibers are mentioned as possible contributory factors [*85*]. Since the heart is viewed as a composite of many units the gradient concept can apply to some of its component parts, and the presence of "local gradients," for example across the thickness of the ventricular muscle, can be considered in an attempt to arrive at a unifying concept of changes in T [*43,47*]. It is not possible, however, to predict mean changes in total gradient of the heart from local differences existing in certain areas, such as the precordium, or vice versa [*1*].

Wilson developed the gradient concept on the basis of the smallest possible units, the assumed differences in the duration of the excited state of two adjacent fibers. Ashman [*16*] and Gardberg

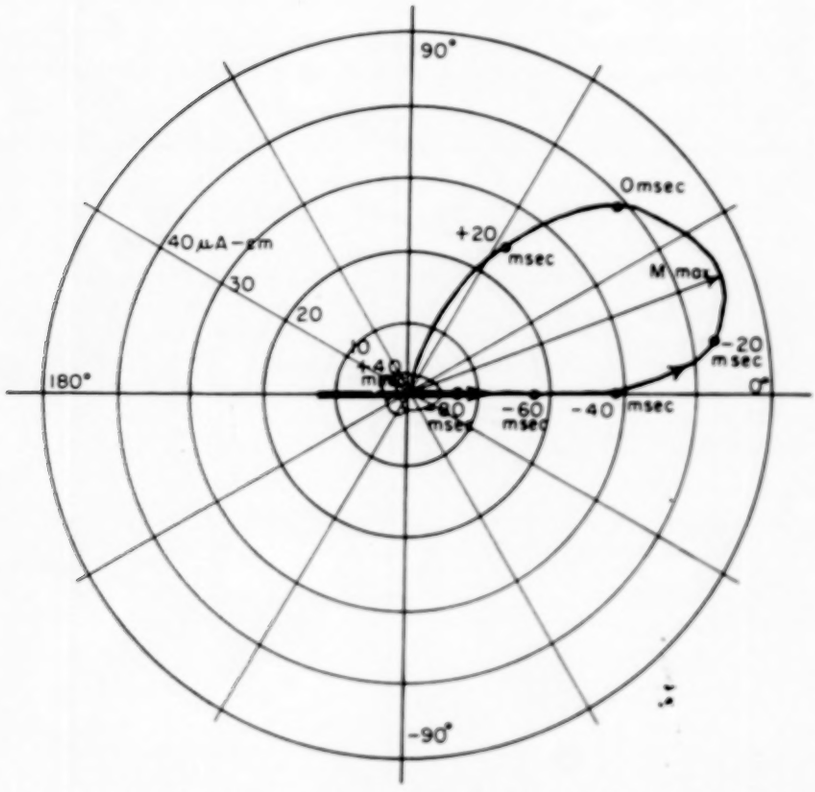


FIG. 14. Ventricular vector loop, frontal plane (bullfrog, not perfused). Strength time course for a dipole moment of frog ventricular muscle whose longitudinal axis is horizontal. 0 time represents peak of QRS. Maximum dipole moment in this instance measured 45μ amp.-cm., and $\theta = 21°$ (from Nelson and Hecht [*75*]). (See text.)

[*41*] have derived a system of vectorial analysis of the heart as a whole from it, extending the two-dimensional aspect to a concept of a spatial ventricular gradient. The actual demonstration that differences in the duration of the excitatory state exist in adjacent myocardial fibers is difficult since the complexities of microelectrode recording multiply when more than one fiber is impaled in a beating heart. Such differences can, however, be demonstrated during perfusion of the heart with various test solutions which slowly diffuse into the inter fiber space, thereby affecting one fiber more than another. Figure 15 presents an experiment using a perfusate with a K^+ content eight times normal: during the infusion changes in the configuration of T and differences in the duration of the plateau of two impaled fibers developed to a striking degree. This proves that it is possible for heart muscle to operate with widely different conductance ratios of one fiber to the next.

The concept of the ventricular gradient is generally invoked to provide a semi-quantitative expression of "primary" T wave changes, i.e., those not obviously the consequence of an altered QRS complex (although changes in QRS may have an influence on the gradient also [*85*]). A statement was made earlier that uniform changes in the recovery process of fibers should change at least the magnitude of T, and modify the RS-T segment. For the effect of digitalis, at least, such interrelationships between simultaneously recorded intracellular and surface potentials have been demonstrated [*111*]. It is of course very likely that neither of the two mechanisms operate independently or exclusively. Figure 16 represents an attempt to combine these effects; uniform changes in slope together with differences in the length of the action potential may account for a variety of quantitative and qualitative alterations of the RS-T segment and the T wave in a surface electrocardiogram. Beyond these somewhat general statements lies a large field of unexplored ground [*47*].

The inhomogeneous volume conductor of finite extent:

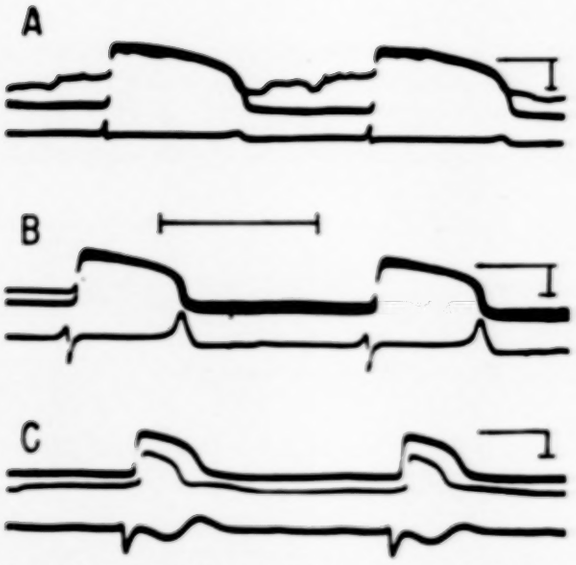


FIG. 15. Perfused heart of bull frog, excised, spontaneously beating. Two fiber impalements, 2 mm. apart, together with surface electrogram. A, control, intracellular potentials superimposed. B, as before, seven minutes after infusion with Tyrode containing five times normal concentration of K^+. C, four minutes after Tyrode solution containing eight times normal K^+. Note shortening of plateau and marked difference in duration of action potential of the two fibers in (C) as compared with (A). B shows K^+ effect on slope of repolarization with little if any difference of the two fibers in duration. It is possible that the classic changes of hyperkalemia observed in the surface record in (B) are primarily the result of changes in slope of repolarization while the advanced changes in (C) may be the result of slope changes and differences in duration of one fiber from the next (effect of "ventricular gradient"). Time line: one second. Zero line and calibration (50 mv.) indicated on the right of electrocardiographic tracing.

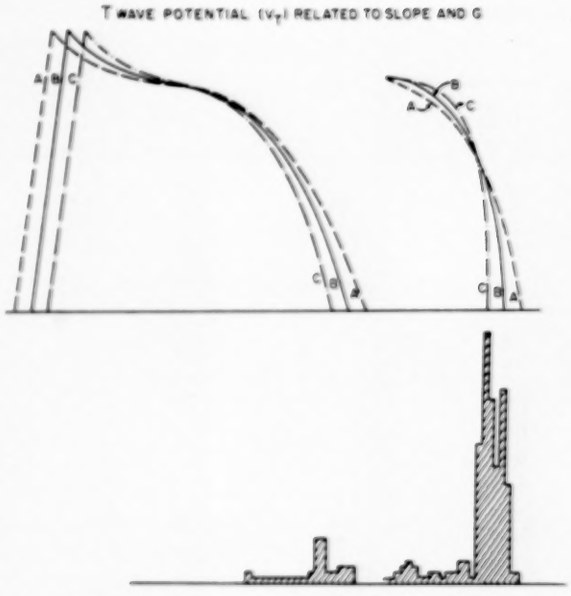


FIG. 16. An attempt to relate magnitude of T to a combination of changes in ventricular gradient and slope of final phase of recovery. Three fibers (A, B and C) show different duration of the excitatory state: (C) is shortest and (A) is longest. Differences in voltage for each time instant are plotted below. Size of T increases sharply if slope is changed while gradient remains unaltered.

It is interesting that the laws which describe the flow of electric currents in an infinite homogeneous medium allow the calculation of an electrocardiographic curve with surprising accuracy considering that they are obtained from an eccentrically located generator of unknown type submerged in a limited, irregularly shaped inhomogeneous body such as the thorax. It is possible to predict the distribution of potential differences on the surface of the torso if the location and strength of the internal generator are known, and if the heart is considered as an "equivalent" single dipole, i.e., the statistical average of any distribution of sources and sinks. The reverse, prediction of the location and magnitude of the generator dipole from surface potentials and, specifically, prediction of true cardiac performance from any surface recording system, is considerably more complex. Helmholtz expressed this clearly in 1853: "a given electromotor surface may be equivalent to an infinite number of systems of internal electromotive forces, all of which have only the property in common, that they may produce equal potentials at the surface of the insulated conductor" [*12*]. This problem has shown little promise of solution, particularly if it is assumed that the heart cannot be considered under all circumstances a single dipolar point source of energy [*3*]. Helmholtz's statement, however, can be challenged at least to some extent by the extensive mathematical analysis of Gabor and Nelson [*40*] who developed a procedure based on vector calculus which consists of integrating the product of the potential obtained from the thorax and a proportionality factor, the unit normals of the surface. These and other analyses since then [*1,3*] are exceedingly laborious but they again indicate that at least calculated and experimental model data agree well enough. From a purely mathematical basis, Martinek and Yeh [*72*] have developed an "interior space theorem" which is alleged to give the desired solution of one surface potential distribution corresponding to one and only one dipole or combination of dipoles or multipoles. Although no experimental

TABLE VI
SPECIFIC RESISTANCE OF BODY FLUIDS AND TISSUES
(Ohm cm.)
(A.C. FREQUENCY 10 TO 1,000 C.P.S.)

Tissue	L*	SK†
Saline solution	50	...
Whole blood	200	...
Heart and blood	250	...
Heart muscle	...	940
Skeletal muscle	600	900
Lung, deflated	400	...
Lung, inflated	750	1,100
Fat	2,000	3,000
Bone	6,000	...

* From LEPESCHKIN, E. Modern Electrocardiography, Baltimore, 1951.

† From SCHWANN, H. P. and KAY, C. F. *Ann. New York Acad. Sc.*, 65: 1007, 1957.

confirmation of this concept is as yet available, it seems possible that a workable solution of the dilemma expressed by Helmholtz may be provided by exact although complicated mathematical treatment. At this stage one may say that to the extent that these calculations agree with model experiments the factors of possible inhomogeneities of body tissues and the boundary limitations of the chest may be of little concern. Some remarks, however, bearing on these particulars are in order.

Inhomogeneities: Observations on electrical resistance of living tissue to an alternating current can be considered a measure of body inhomogeneity. For tissues examined they reveal fairly uniform values of between 500 to 1,000 Ω cm. at frequencies in the range of electrocardiographic complexes (10 to 1,000 c.p.s.) (Table VI) [*18*]. Even inflated lungs have resistance values not much different from those of more solid structures. A plot of isopotential lines radiating from a central dipole to the circular border of an electrolyte tank model of the kind illustrated in Figure 17 shows little distortion even when lung conductance is given a value of ¼ that of the remainder of the tissues of the thorax [*76*]. Fat and bone have high resistivities but in terms of total mass involved these structures play a relatively insignificant part. Capacitance of tissues also is negligible [*87*]. Values obtained earlier for direct current are smaller but again showed little difference between tissues examined [*21a*]. It may be concluded, therefore, that tissues other than blood surrounding the heart are reasonably homogeneous and that the laws discussed herein are applicable.

The heart itself, however, when filled with blood, has a much lower specific resistance than the tissues surrounding it; this is apparently the result of blood contained within its cavities [*20,87*]. Resistivity of the blood is only 20 per cent that of other structures of the thorax. The studies concerned with the influence of such a high internal conductance (shunting) on the externally recorded potentials are therefore of interest [*29,77,78*]. These have demonstrated that with the resistivity of the intracardiac fluid one-fifth that of the external fluid the deflections obtained from the surface appear to be only about 50 to 75 per cent of their true value. Nelson has modified equation 21 correcting for conductivity ratios [*78*]. The potentials of a point outside a disk of conducting material are then given as:

$$V = \frac{2G}{1+G} K' \cdot (e \cdot l) \log_e \frac{r_1}{r_2} \qquad (26)$$

with G the ratio of internal and external resistivities for a two-dimensional system. Additional equations are given for three-dimensional volume conductors. These considerations apply primarily to a tangential spread of excitation while surface potentials from radial dipoles such as intramural spread are unaltered or enhanced. As an example, the progressive increase of R in records successively taken from within outward in ventricular muscle have in part been explained on the basis of gradually lessened influence of the interior shunting effect of the heart [*29*]. The influence of intracardiac blood is therefore complex, particularly in leads close to the heart. In surface records removed from the heart, other factors being equal, the larger the volume of blood contained within the cardiac cavities and the higher the external resistances surrounding the heart, the larger the shunting effects of the heart itself in scaling down QRS complexes, and the smaller the potentials recorded at the thoracic surface. This effect will tend to concentrate the current source and to increase the possibilities of considering the heart as equivalent to a central point source dipole. Since less blood is contained in the ventricular cavities during the inscription of T than during QRS, a correction for the smaller than true magnitude of QRS should enter the calculation of the ventricular gradient [*78*].

The finite boundary: Frank [*39*] has recently given a discussion of general boundary problems which includes an extensive treatment of leads, lead fields, and lead vectors. We shall consider here only the problems of the influence of a finite boundary on the configuration of a recorded surface potential. An experimental approach to this has been available in the form of a circular tank divided by a disk into a top and bottom layer [*18*]. Currents generated in the top layer, instead of being refracted by the tank boundary, flow around the edges of the disk into the bottom layer simulating an "infinite" medium. Figure 17A shows that the mapping of isopotential lines in such a system closely coincides with those obtained by calculation from equation 21. On the other hand, the distortion induced by a finite boundary in a single layer tank is seen in Figure 17B where (a) the field appears distorted, and (b) the magnitude of the potential values is increased.

A solution which allows an estimate of the degree of distortion has been available since 1884 by Thomson (Lord Kelvin) [*14*] who proposed the use of image analysis, an account of which has been reprinted recently [*46*]. This image concept has been referred to by Pruitt and Valencia, Frank, and Nelson [*39a,74,82*]. (It has no relation to the "image surface" of Burger, or to the mirror image concept of cancellation potentials.) Thomson's treatment consists of assuming that the field within a limited boundary is equal to a field in an infinite medium where source and sink remain in their original position but where an imaginary source and sink is added which is the mirror image of the true dipole, the mirror being represented by the boundary. (Fig. 18.) This image dipole may be considered an antiparallel dipole of equal strength and equally distant from the boundary than the actual dipole. For a finite circular disk with the recording point close to the boundary the potentials may be calculated by still another modification of equation 21:

$$V = \frac{2G}{1+G} K' (e \cdot l) \log_e \frac{r_1 r_3}{r_2 r_4} \qquad (27)$$

This equation now describes what is desired, namely the potential variations, V, obtained from a dipole submerged in an inhomogeneous medium of finite extent such as the thorax. All symbols are as before, r_3 and r_4 represent the distance from the recording point to the sink and source images. Additional equations for other finite media and modifications of dipole position and recording points are available [*76*]. These estimates reveal potential differences at P which are considerably in excess of those obtained in an infinite medium. They are in general opposite to the effects of the highly conducting internal cardiac milieu. It is not known to what extent cancellation caused by these opposing factors may occur, and how much this

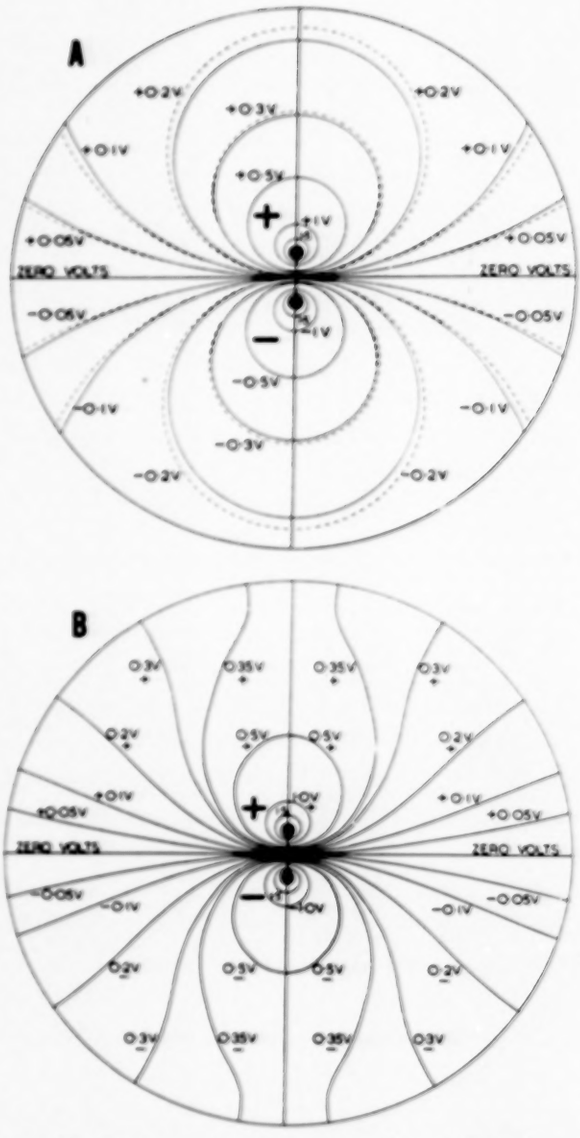


FIG. 17. A, isopotential voltage lines in a double layer tank plotted for an artificial dipole (solid lines). Calculated equipotentials from equation 21 given by dotted line. B, isopotential lines in single layer tank with a dipole of equal strength as in (A). Note distortion by boundary effects, and increase in voltage obtained at the margin of the tank (from Nelson [*74*]).

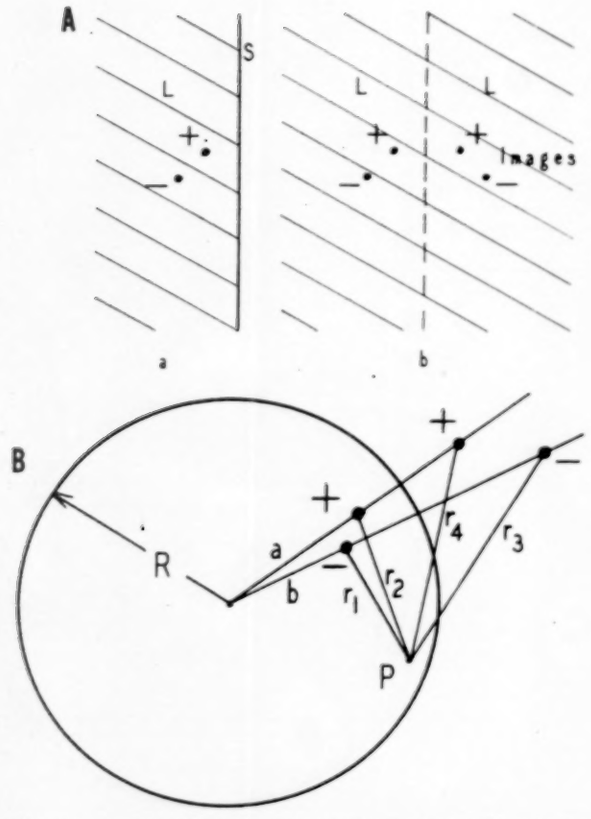


FIG. 18. Aa, source and sink inside a conducting fluid adjacent to a straight insulating boundary. Ab, shows the mathematically equivalent situation: S is removed, the fluid is now considered infinite in extent, and an image source and sink has been added. B, an image system for source and sink in a circular conductor of finite extent. The images are located along the radii extended at a distance R^2/a and R^2/b from the center (from Nelson [74]). (See text.)

might contribute fortuitously to the observed agreement between experimental data and those calculated from equations developed for a homogeneous medium of infinite extent.

CONCLUDING REMARKS

Any attempt to describe the complexities of the biological system of the heart within the chest in terms of known electrical characteristics must remain incomplete. This review has attempted merely to point to certain biophysical concepts that are likely to prove of continuous interest to physicians concerned with interpretation of the electrical phenomena of the heart beat. Many important contributions have been entirely omitted, others appear overly stressed. Thus, for example, lead field concepts, the spread of current through the heart, and studies designed to define an orthogonal reference frame for lead positions have been omitted whereas cellular phenomena of excitation have been stressed more extensively than is usually the case. Field problems, however, may have reached a certain limit for those concerned with practical electrocardiography while the studies on the nature and origin of the electrical events of a cardiac fiber are destined in time to alter and to enlarge our views on the nature of an electrocardiographic curve.

Two general statements can be made: (1) The mathematical treatments relating to the nature and distribution of electrical potentials and currents developed between 1850 and 1900 by Helmholtz, Thomson, Planck, Bernstein, and many others have remained the foundation for a modern electrocardiology; (2) Model experiments and calculations based on these concepts have yielded theoretical action potentials which are nearly identical with those obtained by experimental methods, indicating that present concepts of the nature and distribution of the electrical events of the heart cannot be far off the mark.

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The Regulation of the Performance of the Heart*

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THE objective of this paper is to present as systematically as possible an approach to understanding those variables which normally influence the contraction of the ventricle and the performance of the heart. It will attempt to evaluate the present advantages and limitations of the Frank-Starling mechanism so that the "Law of the Heart" elaborated by Starling a half century ago may be advantageously integrated with the performance of the heart under central nervous system control. Considerable emphasis will be placed on other intrinsic mechanisms which influence ventricular performance, on the significance of changes in the activity of the atrium insofar as these modify the ability of the heart to adapt the organism to varying states of activity, and on a more integrated manner of appreciating the role of the carotid sinus in circulatory regulation.

The history of the waxing and waning of the influence of Starling's Law of the Heart constitutes an interesting chapter in the development of circulatory physiology. His findings were initially greeted with a positive response and were regarded as a true laboratory *tour de force*. As a result, physicians were more inclined to regard the exotic happenings in the cloistered laboratory as being a more substantial source of help in the understanding of their everyday problems than they might previously have supposed. This attitude, however, was gradually replaced by growing disillusionment with the practical usefulness of the concept not only because of an incomplete understanding of it on the part of some who tried to apply it but also because, even when the concept was correctly appreciated, it failed adequately to embrace certain observed circulatory phenomena.

It appears desirable at this time to indicate the manner in which the system-complex under consideration was analyzed and to assign a specific meaning to certain terms used in this study. First, a determination of the conditions under which and the extent to which an organ makes intrinsic adjustments to varying conditions, i.e., autoregulates, is a helpful precursor to an analysis of the effects of extrinsic influences. The term "autoregulation" will be used to describe phenomena occurring in an organ which are not attributable to nervous or chemical influences originating outside that organ and which can reasonably be construed as being of value to the performance of the total organism. The term "myocardial contractility" will be used in a specific manner. When, from any given end diastolic pressure or fiber length, the ventricle produces more external stroke work and more external stroke power (stroke work per systolic second) an increase in ventricular contractility is said to have taken place, and *vice versa*. Implicit in this definition is an increased rate of development of tension when contractility increases. Specifically excluded is any increased work that may be performed as the result of afterload from the same end diastolic length since the rate of development of tension is not increased under such circumstances prior to the application of the afterload. The term "ventricular function curve" (VFC) will be used to designate (1) the relation between mean left atrial pressure and left ventricular stroke work (VFC_{LA}), (2) the relation between left ventricular end diastolic pressure and left ventricular stroke work (VFC_{LA}), and (3) the relation between changes in left ventricular myocardial fiber length and changes in left ventricular stroke work (VFC_{FL}). When the terms "stroke work" and "stroke power" are employed they will always refer to external stroke work and external stroke power. The term "pressure-length relation" will be used to indicate a curve

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describing the relation between changes in the length of a selected segment of left ventricular myocardium and simultaneous changes in left ventricular diastolic pressure [*1*]. The historic development of any particular subject and the methodology and methods of calculation used in the individual experiments discussed will be alluded to only when it seems especially desirable since such information can be obtained in the source material cited [*1–6*].

PERFORMANCE CHARACTERISTICS IN THE ISOLATED HEART (INTRINSIC MECHANISMS)

The Ventricle

Heterometric Autoregulation. Cardiac as well as skeletal muscle will, within certain limits, contract more forcefully from a longer initial length. One type of intrinsic response, therefore, exhibited by the ventricle of the isolated heart is the well known Frank-Starling mechanism. *This endows the ventricles with performance characteristics such that the heart ejects whatever volume is put into it.* For, if inflow is augmented and end diastolic pressure and fiber length are thus increased, the ventricle contracts more forcefully and expels an augmented stroke volume. This occurs on a beat-to-beat basis. Because this basic mechanism employs a change in initial fiber length, it is designated as heterometric autoregulation [*3,6*].

Relation between left ventricular end diastolic pressure (LVEDP), myocardial segment length, and stroke work: The three curves plotted in Figure 1 (top) show certain static variables relevant to a consideration of heterometric autoregulation. Curve A shows the relation between LVEDP and stroke work (VFC_{LV}). Curve B shows the relation of changes in the length of a segment of left ventricular myocardium to changes in LVEDP (pressure-length curve). Curve C shows the relation between changes in ventricular segment length and changes in stroke work (VFC_{FL}). As anticipated from the postmortem ventricular pressure-volume curve [*7,8*], curve C has a more nearly one to one relation than does either curve A relating LVEDP to stroke work or curve B relating LVEDP to fiber length. Such observations support the opinion that fiber length-stroke work relation is the most appropriate and biologically meaningful point of departure in analysis of the control of cardiac function even though, as is often the case, other values may be more conveniently determined experimentally.

The relations expressed by these curves constitute a descriptive analysis of what is meant by heterometric autoregulation. As end diastolic pressure increases there is an increase in stroke work which is large relative to the pressure increase (curve A). If the steep position of the curve is assumed to be that which is normally operative (as for example in the various phases of the respiratory cycle) then large changes in ventricular stroke work can be obtained without extensive changes in the pressure necessary to fill the ventricle or the pressure in the atrium and veins behind it. This fact is facilitated by the upward concavity of curve B, i.e., the relatively large changes in fiber length brought about by small pressure changes in the ventricle on the lower, sensitive portion of this curve.

Changes in myocardial contractility: The lower right panel in Figure 1 shows ventricular function curves obtained from a heart paced at a constant rate. The first curve (C) was obtained before and the second curve (NE) during the administration of norepinephrine. The dashed lines connect the points relating mean left atrial pressure (MLAP) to LV stroke work; the solid lines connect points relating LVEDP to LV stroke work. Both VFC_{LA} and VFC_{LV} are shifted to the left during the administration of norepinephrine. From any given LVEDP, the left ventricle produces not only more stroke work but also more stroke power and, in each instance, high speed tracings reveal that the rate of development of tension is greater from any given LVEDP during the administration of norepinephrine [*4*]. An increase in contractility, therefore, takes place. It was noted that as long as the norepinephrine infusion was maintained the left ventricle continued to exhibit heterometric autoregulation along the curve NE. When the norepinephrine infusion was withdrawn the heart again exhibited heterometric autoregulation along curve C. It is, in fact, fair to say that each heart continually exhibits heterometric autoregulation on one or another of its ventricular function curves. The shift from one curve to another is determined by whether an intervention which can cause such a shift is imposed. Numerous other catecholamines, such as metaraminol [*9*] and mephentermine sulfate [*10*], have also been found to shift the VFC_{LA} to the left even though aortic pressure is held constant and the increase in stroke work is accom-

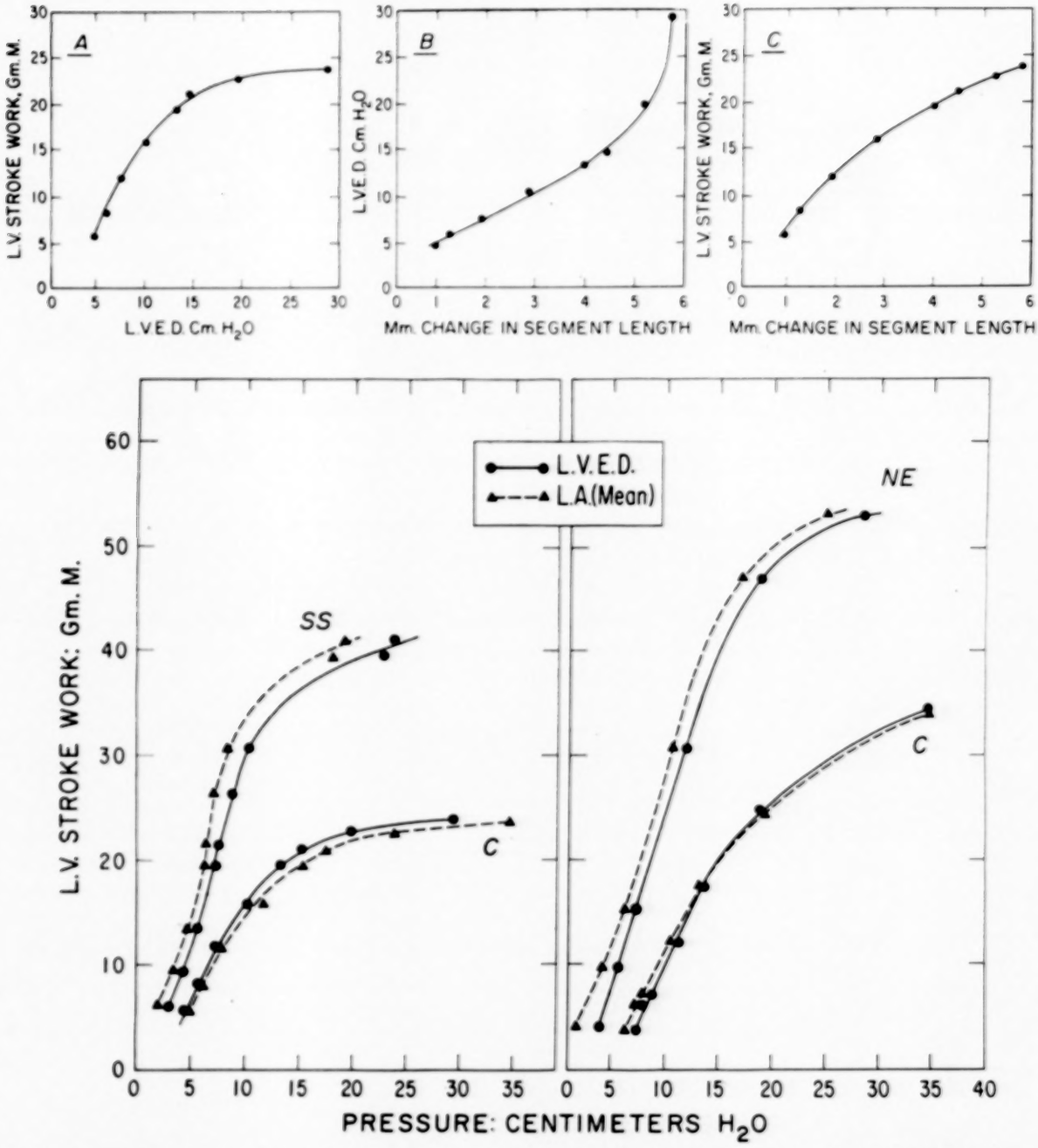


FIG. 1. *Upper*, panel A shows the relation between left ventricular end diastolic pressure and stroke work. Panel B shows the relation between changes in end diastolic myocardial segment length and left ventricular end diastolic pressure. Panel C shows the relation between changes in left ventricular end diastolic myocardial segment length and stroke work. *Lower, left panel*, dashed lines show relation between mean left atrial pressure and left ventricular stroke work during control period (C) and during stellate stimulation (SS). Solid lines show relation between left ventricular end diastolic pressure and stroke work during control period (C) and during stellate stimulation (SS). Bilateral cervical vagotomy; right stellate intact. Heart rate constant at 171 per minute. Dog weight = 17 kg. *Lower right panel*, symbols same as at left except that a continuous infusion of 0.36 gamma per minute of norepinephrine base was administered during curve marked (NE) after control curve (C) was obtained. Bilateral cervical vagotomy; right stellate intact. Heart rate constant at 140 per min. Dog weight = 16 kg.

plished solely by increasing stroke volume. Ventricular function curves as a means of describing alterations in the performance characteristics of the heart appear to have been useful in analyzing the effects of digitalis [*11*], hypothermia [*12*], surgical interventions on the heart [*13,14*], and in evaluating the toxicity produced by agents used to achieve cardiac arrest [*15*].

Homeometric Autoregulation. A second type of autoregulation occurs in the ventricle of the isolated heart. Unlike heterometric autoregulation which occurs immediately, it requires at least several beats to develop fully, and appears after an increase in ventricular activity such as follows an increase in aortic pressure or heart rate. As a result of the ensuing increase in myocardial contractility, the heart maintains either the same LVEDP and fiber length or values more similar to those which obtained prior to the activity increase than would have been possible if this type of autoregulation had not taken place. It is therefore referred to as homeometric autoregulation. As a result of this mechanism *the heart is endowed with performance characteristics such that it can expel the same stroke volume against a wide range of resistances without more than a brief invasion of its heterometric reserve* [*3*].

Hemodynamic factors eliciting homeometric autoregulation: (1) *Aortic pressure (Anrep effect).* The transient phenomena that occur immediately after the increase and subsequent decrease of resistance to left ventricular ejection are shown in the left panel of Figure 2A. There are four distinct phases. In phase 1, immediately after increasing the aortic resistance, LVEDP rises along with the elevation of aortic pressure. The beginning of phase 2 (first arrow) occurs shortly thereafter and is signaled by the decline in LVEDP while aortic pressure continues to rise. This decline in LVEDP continues until the new equilibrium level is reached (phase 3). When the imposed aortic resistance is suddenly removed, LVEDP drops sharply (second arrow) and to the lowest level observed in the sequence (beginning of phase 4); thereafter, in phase 4, it gradually returns to the level which obtained prior to the increase in aortic resistance. During the high resistance period the stroke volume is essentially the same and stroke work doubles without an increase in LVEDP.

Figure 2B shows the range over which these phenomena can occur even when coronary flow is not allowed to vary appreciably as the result of changing aortic pressure. The run labelled I was conducted with mean aortic pressure held approximately constant and stroke work increased by increasing stroke volume as indicated in the bottom two panels. Then, at each of four different stroke volumes (runs II, III, IV and V), stroke work was increased solely by increasing aortic pressure. In runs III, IV and V there was a substantial range of stroke work which could be accomplished with little change in LVEDP. It was of interest to note that the larger the value at which stroke volume was held constant, the steeper was the VFC_{LV} when aortic pressure was increased.

Above a critical level of coronary flow (below which diminished contractility is observed [*16*]) augmenting coronary flow does not increase contractility [*3*]. Further, in experiments in which myocardial segment length was measured simultaneously with LVEDP, no change occurred in myocardial extensibility when aortic resistance was increased [*3*].

Over a certain range, the work produced by muscle will be greater after it encounters an afterload (an increased resistance to shortening at some interval after it has begun to contract from a given initial length) than before [*17,18*]. This is an inherent property of muscle in any given biochemical state and, as far as is known, requires no alteration in its state to exhibit this phenomenon. This may, of course, contribute to the increased work produced in the first beat after an increased aortic resistance is suddenly applied. Homeometric autoregulation is *not* meant to encompass this aspect of cardiac muscle performance, but rather refers to the increased contractility which develops (phase 2) in the subsequent beats after increased resistance is applied. The rate of development of tension cannot be and, in fact, is not increased on the first beat since the ventricle has no way of sensing that it is to encounter an afterload until the aortic diastolic pressure is at least equaled by that in the ventricle. In subsequent beats the external stroke work, stroke power, and rate of development of tension is increased; only then is an increase in contractility said to have taken place [*3*].

(2) *Heart rate (Bowditch effect).* The patterns of response observed after an abrupt change in heart rate without a change in aortic resistance are shown in the right panel of Figure 2A and more particularly in Figure 3A. In the latter, shortly after the increase in rate there is (in

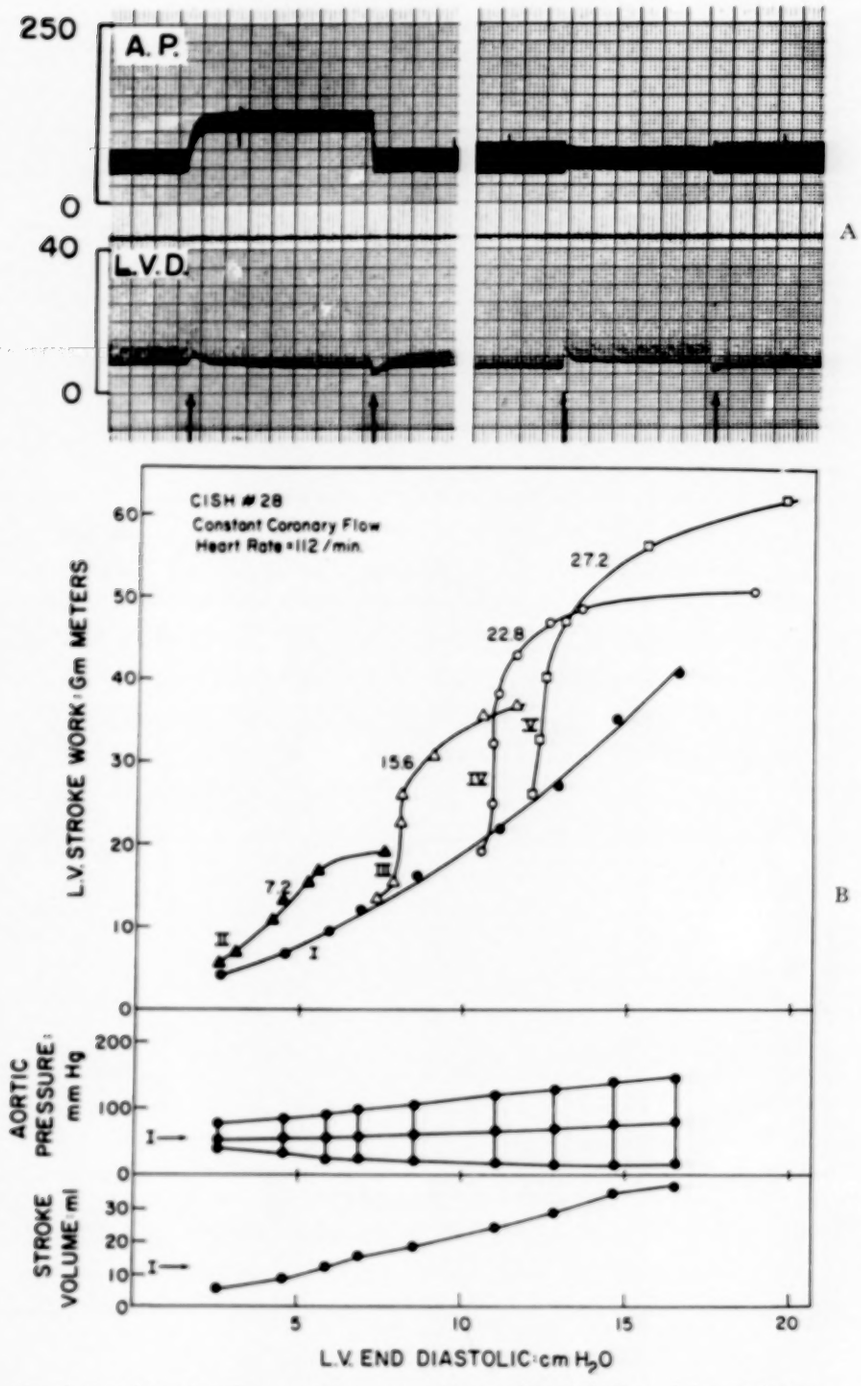


FIG. 2. A, *left panel*, A.P. = aortic pressure in mm. Hg; L.V.D. = left ventricular diastolic pressure in cm. H_2O. Abrupt increase and then decrease in aortic resistance. Stroke volume was 11.1 ml. during low resistance and 10.3 ml. during high resistance. Left coronary flow was constant. Heart rate = 160 per minute. *Right panel*, abrupt change in heart rate from 124 to 163 and back to 131. Cardiac output and total coronary flow held constant. Left and right panels separated by an interval of four minutes. Chart speed = 0.5 mm. per second. B, See text. Figures to left of the curves in upper panel indicate the stroke volumes obtained.

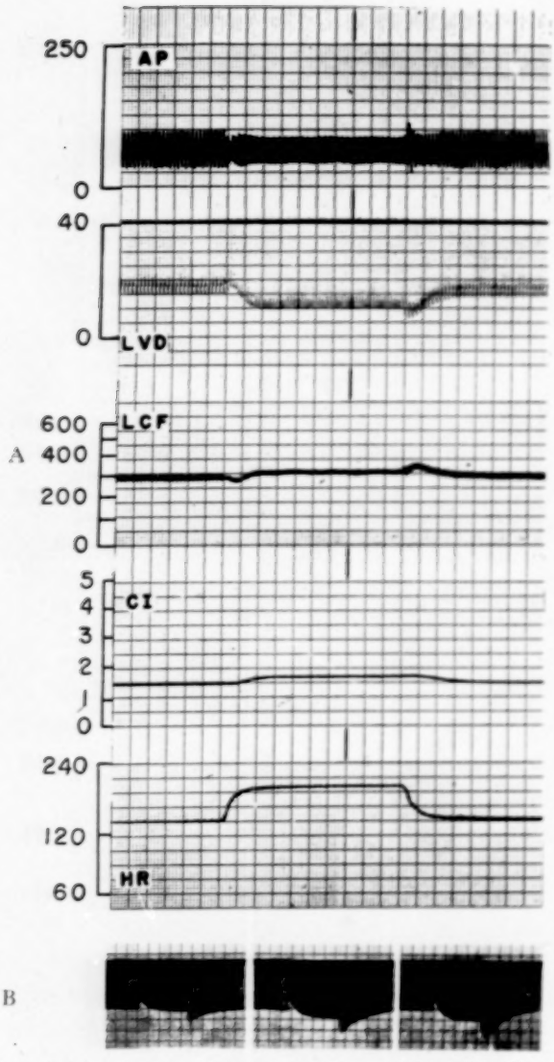


FIG. 3. A, A.P. = aortic pressure in mm. Hg; L.V.D. = left ventricular diastolic pressure in cm. H_2O; L.C.F. = left coronary flow in milliliters per minute; C.I. = cardiac (left ventricular) inflow in liters per minute; HR = heart rate. Chart speed = 2.5 mm. per second. B, isolated rat heart muscle preparation. Rate changed from 30 to 60 to 30 in left panel, 30 to 90 to 30 in middle panel and 30 to 108 to 30 in right panel. Chart speed = 0.5 mm. per second.

phase 1) a rise in LVEDP similar to that which occurs when the aortic resistance is increased. The first few beats after the increase in rate are weaker than the subsequent ones, as evidenced by the aortic pressure tracing. This pattern indicates a diminished cardiac output while inflow remains constant, thus demonstrating that the rise in LVEDP is paralleled by an increase in ventricular volume. At the onset of phase 2, LVEDP declines while the aortic pulse is augmented briefly and a new equilibrium state is reached (phase 3) during which there is a slightly higher cardiac inflow and, presumably therefore, a higher cardiac output. At the onset of phase 4, when returning to the initial rate, it is clear from the lower LVEDP, wider aortic pulse, and maintained inflow that an increase in contractility had taken place during the period of increased heart rate.

Depending on the initial heart rate, the state of the heart and the stroke volume, the LVEDP may not return to or below its initial level during the interval when heart rate is increased. (Figure 2A, right panel.) However, the stigmata of increasing contractility (the decline of LVEDP during phase 2) and of increased contractility (LVEDP lower than control value at beginning of phase 4) were always observed when a period of relative tachycardia was imposed. Further, the changes observed were a function of the extent of the increase in heart rate imposed [3]. Figure 3B shows the change in isometrically developed tension resulting from changing the stimulus rate in a preparation consisting of a strip of rat right ventricle in oxygenated Krebs solution. Since resting tension did not change, an increase in contractility is indicated by a greater downward deflection. In the tracing shown in Figure 3B the rate was changed from 30 to 60 (left), 30 to 90 (middle) and 30 to 108 (right) per minute; in each instance the rate was then promptly returned to 30 per minute. The pattern of changes observed in Figures 3A and 3B are similar in that an increased contractility had been induced in both by an increased rate.

(3) *Stroke volume.* Unlike an increase in aortic resistance or of heart rate, an abrupt increase in stroke volume produces relatively little evidence of homeometric autoregulation [3].

Possible mechanisms involved in homeometric Autoregulation: (1) *Tension time index.* Rosenblueth et al. [19] stressed "the influence of previous activity" on the contractility of the right ventricle. They state "We suggest that whenever the work of the heart increases, this increment determines a further increase in the subsequent contractions and this influence is important enough to overcome that of the initial volume or length," and, "Any increase of work augments the amplitude of the following contractions." Subsequently, however, it was shown that the homeometric influence on the contractility of the ventricle was related to the manner in

which its activity was increased rather than to the increase in work *per se*. The homeometric effect is much more pronounced when aortic pressure is increased than when flow is increased [3]. Further, it was possible to induce evidence of pronounced homeometric autoregulation in the heart even when stroke work was decreased, as shown in those experiments in which pressure was abruptly increased and flow decreased more, so that a fall in stroke work occurred [3].

An analysis of the hemodynamic variables influencing oxygen consumption of the heart revealed that the variable which most closely correlates with myocardial qO_2 is the amount of tension developed by the myocardium as indicated by the area under the systolic portion of the pressure curve per minute = the tension time index [20]. In these studies an increase in aortic pressure or heart rate required the heart to produce a large increase in the total tension developed per minute whereas greater relative increase in stroke volume, with mean aortic pressure and heart rate held constant, resulted in a much smaller augmentation of the total tension developed. As shown herein, increases in rate or aortic pressure produced a marked homeometric influence; with the augmentation of stroke volume this was less well developed. It does not seem unreasonable to suggest, therefore, that an increase in the amount of tension developed by the myocardium per unit of time may be the hemodynamic factor which elicits homeometric autoregulation.

(2) *Ionic concentration changes.* Bowditch in 1871 described phenomena in the frog heart which he referred to as "treppe" or staircase [21]. Shortly after the onset of stimulation the heart exhibited successively stronger contractions with each succeeding stimulus until, several beats later, a plateau was reached; subsequent increases in rate produced the same effect. A common variant of the Bowditch staircase is shown in Figure 3B; after the change of rate in the isolated rat ventricle strip, an increase in contractility became apparent, i.e., a stronger contraction from the same initial tension. The greater the rate change imposed, the greater the increase in contractility at the new rate and the stronger the first few contractions after the return to the control rate. The similarity between the pattern observed in Figure 3B and that in Figure 3A supports the position put forth by Rosenblueth et al. [22] that the Bowditch staircase effect is operative in the adequately supported canine heart; this position is consonant with the experiments of Braunwald et al. [23] in which it was observed that the higher the heart rate the shorter the period of time required for the ventricle to eject a given volume.

Bowditch suggested that each contraction leaves behind it a more favorable state for the ensuing contraction and thus, the higher the rate, the more favorable the condition for contracting. The available evidence relating to the biochemical mechanism by which this takes place can be found in the recent review of Hajdu and Leonard [24], and indicates that potassium leaves the myocardial cell with each contraction and re-enters it in the interval between contractions. Thus, the higher the rate and the shorter the period of diastole, the less opportunity for re-entry of potassium relative to efflux and the lower the intracellular potassium in the new equilibrium state, a condition known to increase contractility [24]. It has, in fact, recently been demonstrated [25,26] that a potassium loss occurs in the perfused canine heart when tachycardia is imposed.

A certain similarity is to be noted between the hemodynamic pattern elicited when activity is increased by either increasing heart rate or aortic pressure. (Fig. 2A.) In both, phase 2 shows an increase in contractility soon after beginning the intervention; in both, phase 4 shows an increased contractility during the intervention. This similarity of patterns suggests that changes in intracellular ionic concentration may occur as a result of the change in the character of the contractions as well as in the length of the interval between them. This view presupposes that a more forceful contraction can either increase ionic efflux during the contraction or, perhaps less likely, so predispose the membrane as to alter net ionic flux in any given time interval between contractions. In any case it now seems clear that, over a wide range, *an increase in the amount of tension developed by ventricular myocardium can produce a biochemical rearrangement which leaves behind it a more favorable condition for subsequent contractions*, and that this phenomenon is not wholly dependent upon an increased coronary flow.

(3) *Norepinephrine.* In any given isolated heart preparation, an aortic resistance can be selected against which the ventricle is unable to eject the same or nearly the same stroke volume without requiring an elevated LVEDP. During

the administration of norepinephrine the same increase in aortic resistance does not require an increase in LVEDP. In some instances a heart which was in relatively poor condition, as indicated by its LVEDP, would show little or sometimes no apparent homeometric autoregulation; when norepinephrine was infused, however, a response could be obtained. Further, the higher the level of circulating norepinephrine, the greater was the increased resistance against which the ventricle could eject a comparable stroke volume without an elevated LVEDP. Lastly, norepinephrine influenced the myocardium in such a manner that it would respond to a sudden increase in pressure not only adequately but also more rapidly. Such findings are not only consonant with the observation that norepinephrine shifts the ventricular function curve to the left and makes it steeper (Fig. 1, lower) but also invite consideration of the possibility that increased ventricular activity (tension developed) either increases the locally available myocardial norepinephrine or facilitates its utilization during homeometric autoregulation. These observations on the effect of norepinephrine [*3*] are in accord with those of Anrep [*27*], but they are not in agreement with those of Rosenbleuth et al. [*19*] in that the level of catecholamine present appeared to be of importance to the exhibition of homeometric autoregulation by the ventricle.

Significance of homeometric autoregulation: Whatever the mechanism or mechanisms by which homeometric autoregulation is accomplished, the resulting increase in contractility has two important consequences: (1) It permits the ventricle beating at any given rate to eject the same stroke volume against a wide range of resistances without requiring an increased LVEDP or fiber length. Thus over certain ranges it acts in such a manner as to conserve heterometric autoregulation for changes in stroke volume. (Fig. 2.) (2) The increase in contractility, especially that aspect of it which is exhibited as a more rapidly developed ventricular pressure, diminishes the proportion of the total cardiac cycle that systole would otherwise require. This is a particular advantage when heart rate is increased since, if such a phenomenon did not occur, the diastolic interval would be so constrained that ventricular relaxation would be incomplete before the next systole [*2*], ventricular filling would be impaired, and coronary flow limited. Of particular interest in this connection is the frequency with which the heart, when its rate was suddenly increased, exhibited *pulsus alternans* in the first few beats but became regular again as the increase in contractility became manifest. (Fig. 3A.)

Influence of Oxygen Availability on Ventricular Performance. The ventricle will produce less external stroke work from any given MLAP when the blood flow to the myocardium is unduly restricted (shift of VFC_{LA} to the right). Under these circumstances when mean atrial pressure rises, LVEDP also rises. As might be expected, the divergence between a control VFC and one obtained during restricted coronary blood flow is larger at the higher filling pressures and stroke works; with more severe degrees of coronary blood flow restriction not only will the plateau of the curve be lower but a descending limb characteristic of the failing heart will also appear [*16*]. With such technics it has also been possible to demonstrate acutely induced unilateral ventricular failure [*16*].

That anemia also shifts VFC_{LA} to the right despite the presence of a higher coronary blood flow is evidence that, during coronary flow restriction, a major influence producing the depressed function is the limited availability of oxygen, rather than the accumulation of metabolites [*28*].

The Atrium

Heterometric Autoregulation in the Atrium. The studies of Blinks, who used a more highly refined technic for studying atrial contractility than had previously been available, have dissolved any possible reservations as to whether or not the force of atrial contraction is a function of its end diastolic pressure and volume (fiber length), as is the case with the ventricle [*29*]. He further demonstrated a shift of the atrial function curve to the left under the influence of catecholamines, and that, while under this influence, a change in atrial distensibility did not take place [*30*]. Acceptable evidence for the presence or absence of homeometric autoregulation in the atrium is not presently available.

Effect of Atrial Systole on LVEDP and Fiber Length. The significance of atrial systole for ventricular filling was first shown by Harvey [*31*] and later by Gesell [*32*] and Wiggers and Katz [*33*]. Recently it has become possible not only to make a more definitive analysis of the influence of atrial systole but also to designate those circumstances under which atrial systole will

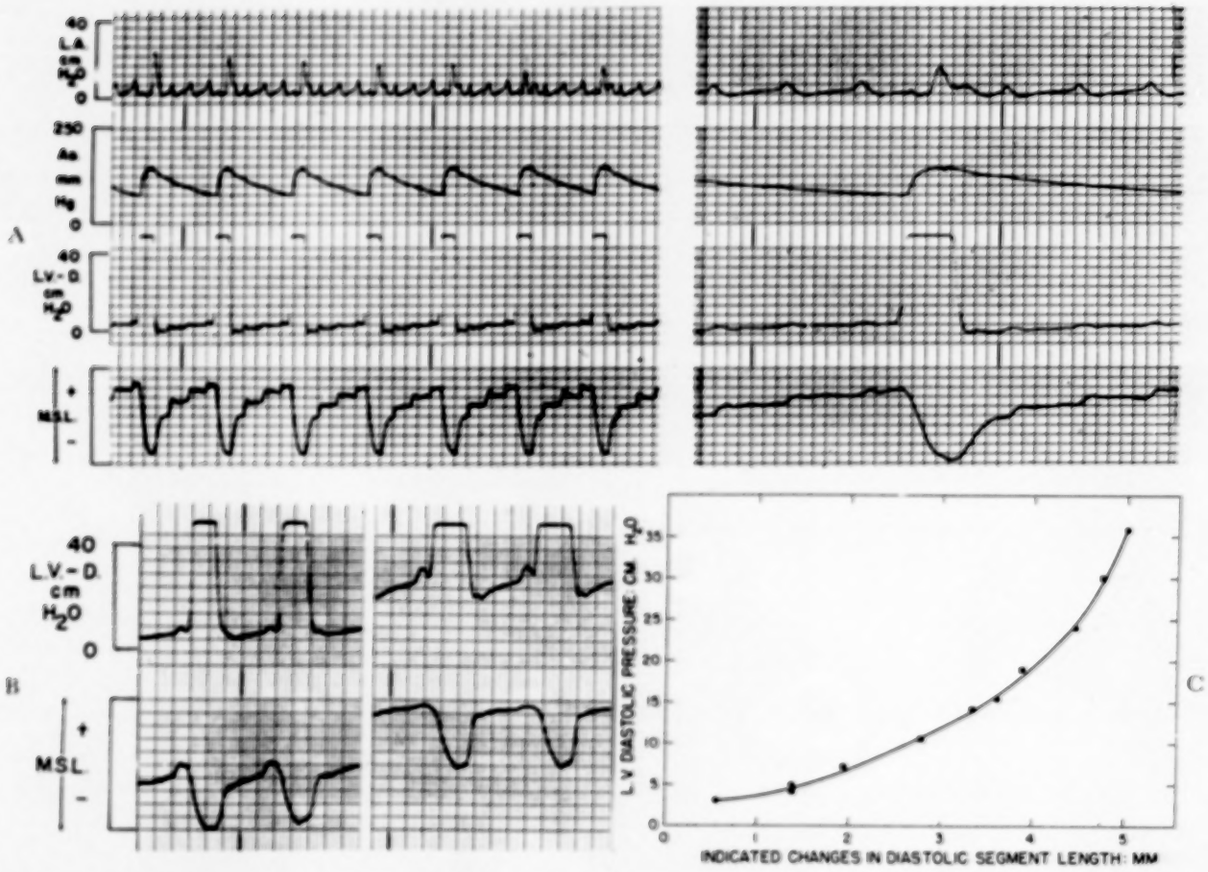
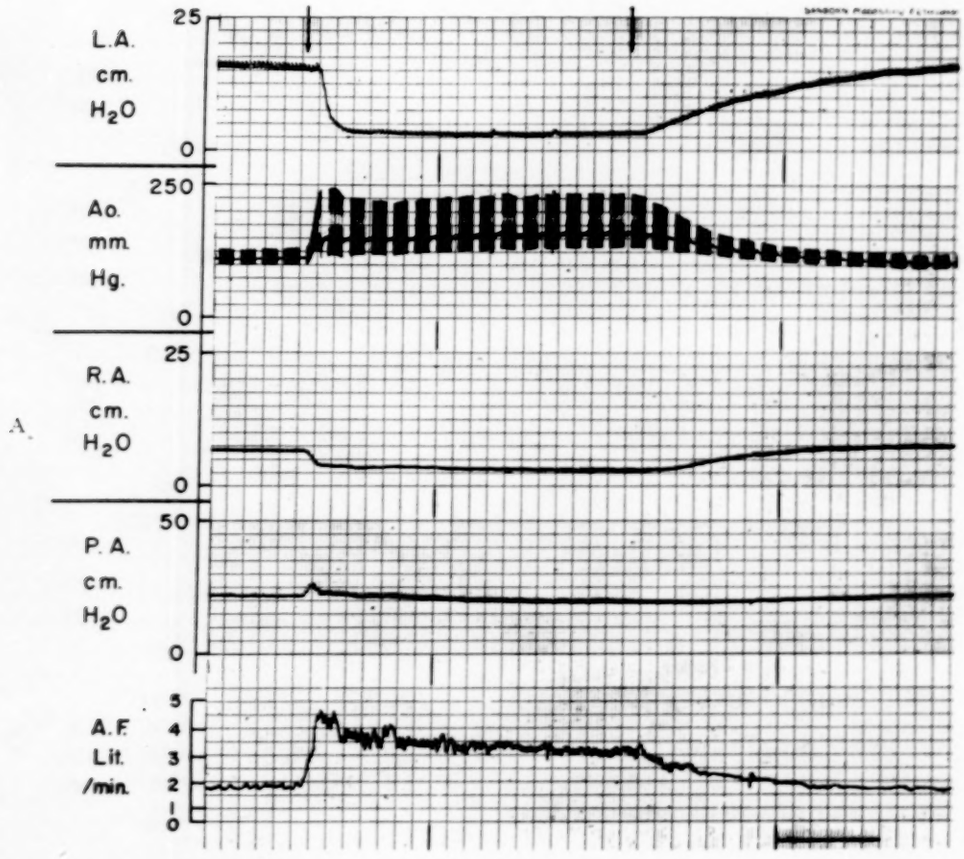


FIG. 4. A, dog with surgically induced heart block. L.A. = left atrial pressure; Ao. = Aortic pressure; L.V.-D. = left ventricular diastolic pressure; M.S.L. = changes in myocardial segment length; + = elongation; − = shortening. Chart speed at left is 25 mm. per second; at right is 100 mm. per second. Ventricular rate = 50 per minute. Atrial rate (paced) = 200 per minute. Note rise in left ventricular diastolic pressure and increased myocardial segment length consequent to each atrial systole. B, L.V.-D. = left ventricular diastolic pressure; M.S.L. = changes in myocardial segment length; + = elongation; − = shortening. Two beats at the beginning and two beats at the end of a rapid infusion of blood. Fifteen seconds between the left and right tracing. Chart speed = 100 mm. per second. C, two successive runs (circles and squares) in which left ventricular diastolic pressure was elevated by the stepwise infusion of blood. The plot shows the relation between diastolic pressure and changes in myocardial segment length during diastasis (prior to atrial systole).

produce more or less diastolic lengthening of the ventricular myocardium [*1*]. The extent to which atrial systole can contribute to the lengthening of the ventricular myocardial fibers can be seen in Figure 4A. In this record, obtained from a dog with heart block, there are four atrial contractions for each ventricular contraction; the results of each atrial contraction can be readily observed in the absence of disturbances produced by ventricular activity. Each atrial contraction, which produced only a small rise in diastolic pressure in the ventricle, caused a substantial increase in myocardial segment length. The observed increases in segment length thus induced were an appreciable proportion of the total segment shortening which took place during systole.

The extent to which the level of ventricular diastolic pressure will modify myocardial elongation due to atrial systole is demonstrated in Figure 4B, which shows two beats at the beginning and two beats at the end of a rapid infusion of blood. In the first two beats, when ventricular diastolic pressure was low, the small increment in end diastolic pressure consequent to atrial systole was accompanied by a substantial segment length elongation. In the last two beats, when the ventricular diastolic pressure was high, atrial systole causes a greater rise in pressure but a much diminished increase in segment



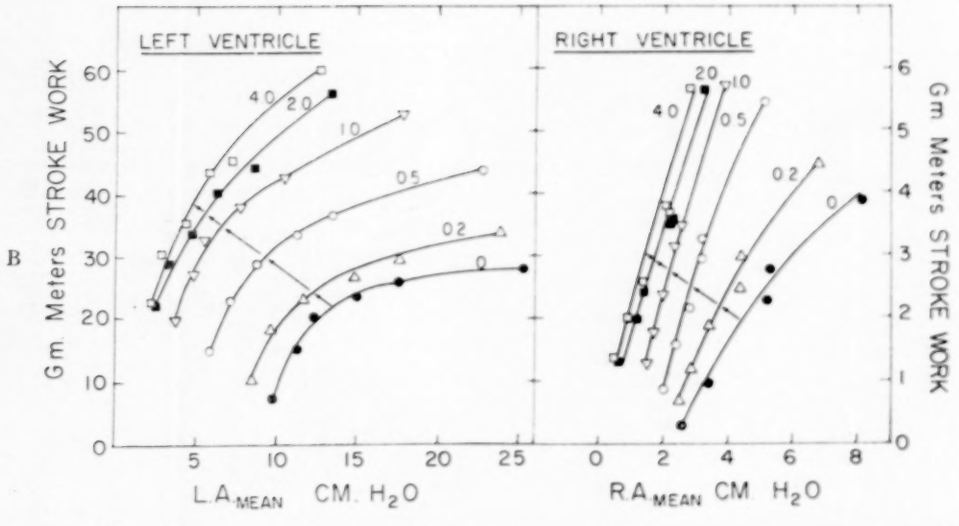


FIG. 5. A, L.A. = mean atrial pressure; Ao. = aortic pressure; R.A. = mean right atrial pressure; P.A. = mean pulmonary artery pressure; A.F. = total aortic flow. Line at bottom right = interval of one minute. During the interval between the arrows at the top of first channel the isolated left stellate ganglion was stimulated at 6 volts, 4 per second with a 5 msec. impulse duration. Heart rate was maintained at 150 per minute throughout by atrial pacing. The rami to the right stellate ganglion were cut and both vagi sectioned in the neck. B, in the left panel are curves showing the relation between mean

length. This relation is expressed in the pressure-length curve of the ventricle. (Fig. 4C.) When the diastolic pressure in the ventricle is low, a small increment in pressure produces a relatively large increase in myocardial segment length (sensitive part). Conversely, at the higher ventricular diastolic pressures only small increases in segment length are produced by a similar or even greater pressure increment (insensitive part).

The extent to which atrial systole can contribute to the end diastolic elongation of ventricular myocardium, when coupled with the dependence of the ventricle's stroke work on initial fiber length, indicates the substantial extent to which variations in the vigor of atrial systole can provide the stimulus for greater or lesser amounts of ventricular stroke work.

NEURONAL EFFECTS ON THE PERFORMANCE CHARACTERISTICS OF THE HEART (EXTRINSIC INFLUENCES)

Influence of Cardiac Sympathetic Nerve Stimulation on the Ventricle. Contractility: Supramaximal stimulation of the isolated left stellate ganglion, while the heart rate is held constant, produces the changes shown in the upper portion of Figure 5. The prompt elevation of cardiac output and systolic, mean, and diastolic aortic pressures is accompanied by a fall in mean left and right atrial pressures, and a widening of the PA-LA pressure difference. The fall of MLAP during stimulation of the stellate ganglion is consistently accompanied by a lowering of LVEDP. The more rapid development of tension, the more rapid myocardial shortening, the elevated aortic pressure, the shorter duration of ejection, and the more rapid relaxation are consistent and noteworthy [*4*]. Changes of the type observed can be seen in Figure 7.

The left panel of Figure 1 (lower) shows data from an experiment in which both LVEDP and MLAP were recorded before and during stimulation of the left stellate ganglion. During stimulation, a shift of the ventricular function curve to the left is observed when either MLAP or LVEDP is plotted against stroke work, just as when norepinephrine is administered. (Fig. 1, lower.) The shortening of systole observed during stellate stimulation, while the ventricle produces increased work from any given end diastolic pressure, indicates a greater increase in stroke power than in stroke work.

The plots of MLAP against left ventricular stroke work (VFC_{LA}) resulting from frequency-graded stimulation of the left stellate ganglion is shown in Figure 5B. The number adjacent to each curve indicates the stimulus frequency used. Worthy of note is the magnitude of the changes in ventricular work produced from a given mean left atrial pressure under the influence of left stellate stimulation (especially since only a portion of the cardiac sympathetic nerves were stimulated) and also the systematic manner in which the position of the curve shifts with the change in the frequency of cardiac sympathetic nerve stimulation. The effects of graded left stellate stimulation on the performance of the right ventricle (VFC_{RA}) are similar to the effects observed on the left ventricle. (Fig. 5B.)

It has recently been demonstrated that such a graded increase in the frequency of cardiac sympathetic nerve stimulation produces a graded increase both in the coronary venous concentration and total efflux of catecholamine from the heart [*34*]. It does not seem unreasonable to assume that there is a similar directional variation of catecholamine concentration in the myocardium as the stimulation frequency is increased.

The possible influence on VFC_{LV} of the degree of synchronicity with which the fibers of the ventricle contract before and during sympathetic stimulation is discussed elsewhere [*4,6*].

End diastolic pressure-length relation: The administration of catecholamines or stimulation of the cardiac sympathetic nerve increases the vigor of ventricular contraction and under these influences the ventricle will contract more forcefully from any given MLAP or LVEDP. (Figs. 1 and 5.) The early studies of Anrep [*27*] and other more recent studies [*4,8,35–37*] indicate that a changed myocardial extensibility cannot, of itself, account for the increased ventricular contraction. However, whether the augmented

left atrial pressure and left ventricular stroke work during control period (o) and during stimulation of isolated left stellate ganglion at 0.2, 0.5, 1, 2, and 4 per second, using 7 volts and an impulse duration of 10 msec. Rami to right stellate ganglion and both vagi sectioned. Heart rate held constant at 150 per minute. In the right panel are curves showing the relation between mean right atrial pressure and right ventricular stroke work in the same experiment.

stroke work is produced solely by a more forceful contraction from a given end diastolic fiber length, or whether a change in the relation between ventricular end diastolic pressure and fiber length contributes to the observed augmentation, has only recently been established [*2*]. At the heart rates studied the relation between LVEDP and segment length is unmodified by stellate stimulation under an intensity of stimulation which markedly increases contractility, as seen in Figure 6. This shows (1) the relation between LVEDP and LV stroke work, (2) the relation between changes in LVEDP and changes in myocardial segment length, and (3) the work produced from any given myocardial segment length, before and during sympathetic stimulation. The absence of any change in the relation between LVEDP and fiber length during sympathetic stimulation shows that under such circumstances the ventricle contracts more forcefully from any given fiber length as well as from any given LVEDP. (Fig. 6, right.)

More complete systolic emptying from any given end diastolic pressure or length during stimulation of the stellate ganglion is evident from these data. Sympathetic stimulation induces delivery of a substantially greater stroke volume (as well as stroke work) from any given left ventricular end diastolic segment length or pressure. Further, the more complete systolic emptying is accomplished in a shorter period of time.

From these data it is apparent that myocardial extensibility [*2*] is not altered by sympathetic nerve stimulation, a position confirmed by more recent findings in the isovolumetric ventricle [*38*]. At heart rates and stroke volumes which leave insufficient time for ventricular relaxation, however, a diastolic "stiffening" of the ventricle will occur [*2,39*]. It was, therefore, of considerable interest to note the consistency with which, at any given heart rate, stellate stimulation provided for the earlier onset of ventricular relaxation as the result of a shortened systole. This is shown in Figure 7, which indicates the importance of this phenomenon. With the heart deprived of sympathetic efferents from both stellate ganglia and paced at a rate of 201 per minute, diastole was limited to 69 milliseconds of a total cycle time of 296 milliseconds. Stimulation of the left stellate ganglion not only increased the force of contraction from a lower end diastolic pressure but also doubled diastolic time, thus increasing the time available for more complete ventricular relaxation. This effect of sympathetic impulses provides means whereby the "normal" ventricular pressure-length relation is more likely to be retained at high heart rates.

The Influence of Efferent Vagal Nerve Stimulation on the Ventricle. When a high intensity and frequency of vagal nerve stimulation is applied to a heart with rate held constant by pacing, no effect on ventricular contractility is observed, e.g., VFC_{LV} is unchanged as seen in Figure 8A [*4*].

Influence of Cardiac Sympathetic Nerve Stimulation on the Atrium. Evidence has been obtained which indicates that the atrium as well as the ventricle contracts with more vigor during sympathetic nerve stimulation. The augmentation of atrial systole in the dog, whether with surgically induced heart block or in the presence of a normal conduction pathway, is shown in the upper and middle set of tracings of Figure 9. In the latter, during stimulation of the stellate ganglion the atrial contraction produces a larger end diastolic increment of both ventricular pressure and segment length. The greater rise in end diastolic ventricular pressure can be attributed only to the more vigorous atrial contraction; the greater elongation of the myocardial segment is attributable not only to the more forceful atrial contraction but also to the lower position on the pressure-length curve of the ventricle (Fig. 4) brought about by the more complete systolic emptying in the previous beat.

The Influence of Efferent Vagal Nerve Stimulation on the Atrium. Stimulation of the distal cut end of either vagus nerve strongly depresses atrial contraction, whether the heart is paced or unpaced, and in the absence or presence of heart block. An example of the latter is shown in the lower portion of Figure 9. The marked diminution of atrial systole results not only in a diminished "a" wave but also in a smaller rise of ventricular diastolic pressure due to each atrial systole. During vagal stimulation MLAP rose while LVEDP fell.

The Transport Function of the Atrium. The extent to which changes in atrial activity can contribute to and vary ventricular filling has already been described. There are, however, other relevant and important considerations. In the circulation the atrium performs a function much like a booster pump; it augments the transfer of fluid from the feed line into the primary or power generating element of the system. There

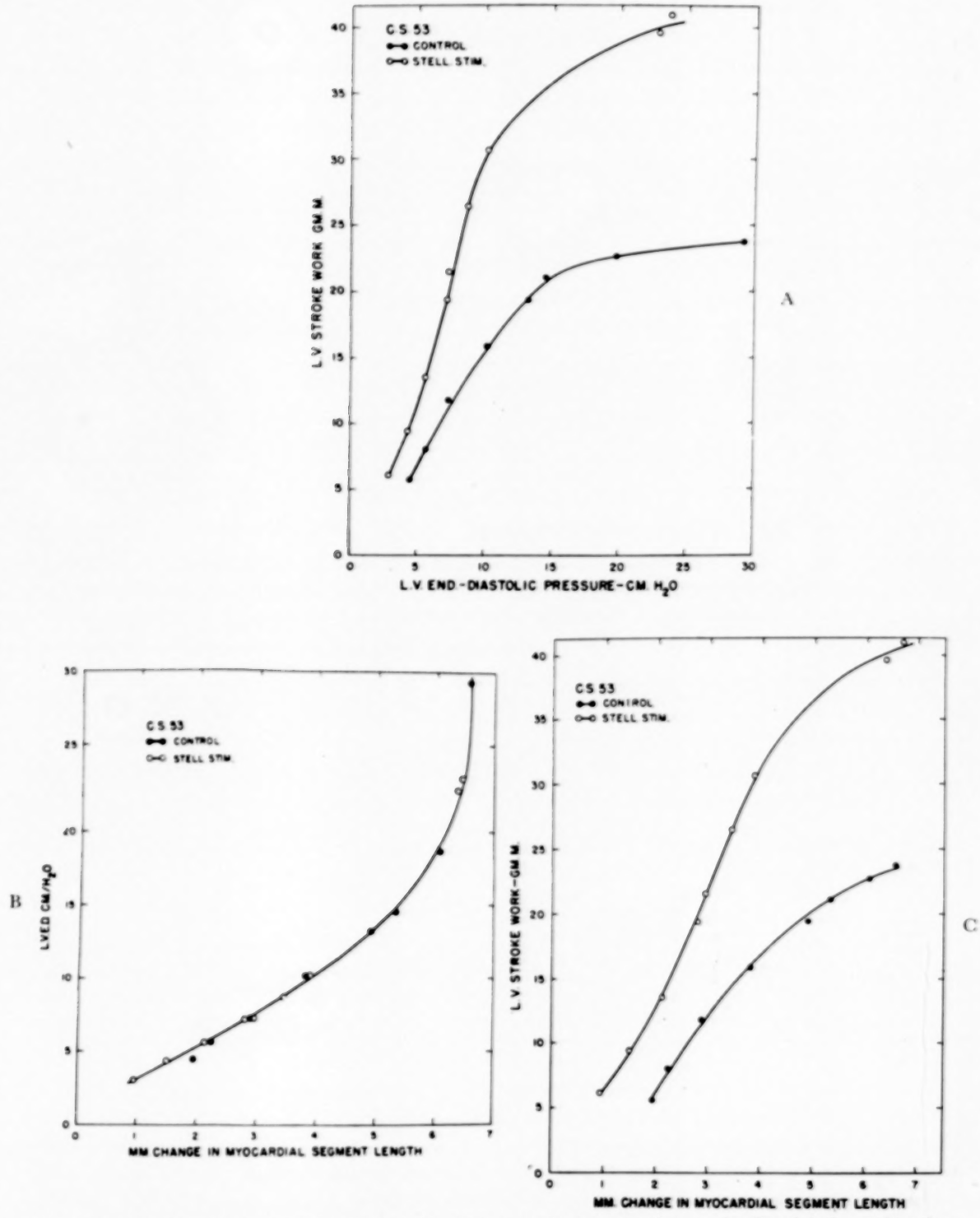


FIG. 6. Data for the curves shown in panels A, B and C were obtained simultaneously. Panel A shows the relation between left ventricular and diastolic pressure and stroke work before (solid circles) and during (open circles) stellate ganglion stimulation. Panel B shows the relation between left ventricular end diastolic pressure and changes in end diastolic myocardial segment length before (solid circles) and during (open circles) stellate stimulation. Panel C shows relation between changes in end diastolic myocardial segment length and left ventricular stroke work before (solid circles) and during (open circles) stellate stimulation. Heart rate held constant at 171 per minute by atrial pacing. Bilateral cervical vagotomy; right stellate ganglion intact.

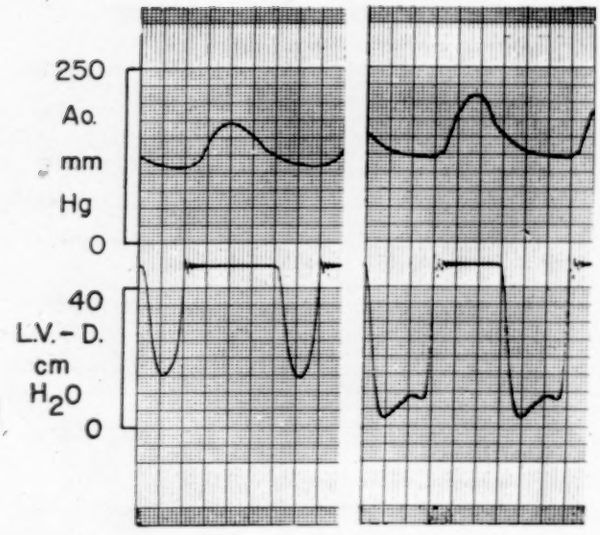


FIG. 7. Atrium paced at rate of 201 per minute throughout. Stellate ganglia isolated. Tracing at left is control. Mean aortic pressure was 138 mm. Hg; stroke volume 9.2 ml.; and L.V. stroke work 17.3 gm. M. Tracing at right taken during stellate stimulation. Mean aortic pressure was 155 mm. Hg; stroke volume 12.4 ml. and left ventricular stroke work 26.1 gm. M. Note lengthening of diastolic interval during stellate stimulation. Chart speed = 100 mm. per second.

are two main consequences when the action of a properly timed booster pump is increased or decreased. The first is that more or less fluid will be forwarded into the main pump, provided, of course, that the latter does not have a stop which limits input. The second is that for a given input into the main pump the pressure in the feed line behind the booster pump will be lower when the activity of the booster pump is increased and higher when the booster pump activity is diminished. Since hemodynamic phenomena in the atrium relate both to that portion of the circulation from which it receives blood and to that part of the circulation into which it forwards blood, the relation between MLAP and LVEDP before and during autonomic nerve stimulation is informative; this is shown in Figure 10. During efferent vagal nerve stimulation the MLAP is higher for any given LVEDP than in the control run. Conversely, during cardiac sympathetic nerve stimulation the MLAP is lower for any given LVEDP than it is in the control run. These differences are more marked at higher stroke volumes [*40*].

The importance of these considerations is exemplified by the plot of MLAP against cardiac output. (Fig. 8C.) When the transport function of the atrium was inhibited by stimulation of the vagal nerve, MLAP had to be 2 cm. H_2O higher in order to maintain a cardiac output of 1 L. per minute. At 2 L. per minute it had to be 5 cm. H_2O higher and at 3 L. per minute it had to be 11 cm. H_2O higher. The fact that stimulation of the efferent vagal nerve had no effect on the performance characteristics of the ventricle in this experiment is shown in Figure 8A) i.e., the relation between LVEDP and stroke work was unchanged during stimulation of the efferent vagal nerve. However, the plot of MLAP against stroke work was shifted to the right during stimulation of the efferent vagal nerve. (Fig. 8B.) It is clear that, whereas the relation between LVEDP and stroke work is determined only by the performance characteristics of the ventricle, the relation between MLAP and stroke work is determined by the performance characteristics of both the ventricle and atrium.

The experimental demonstration that a central venous pressure can be substantially elevated relative to left ventricular end diastolic pressure, stroke work and cardiac output solely as the result of depressed atrial contractility, when taken together with the occurrence of observable atrial pathology in some types of cardiac disease, invites consideration of the possibility that atrial failure may play a role in the genesis of the venous hypertension observed in congestive failure [*40*]. The hemodynamic alterations observed with the acute onset of atrial fibrillation are consonant with this position.

The Influence of Changes in Atrial Contractility on Mitral Valve Closure. It has recently been shown in the dog with heart block that varying the vigor of atrial systole with either vagal or sympathetic stimulation can either promote or abolish closure of the mitral valve prior to the onset of ventricular systole [*41*]. When the pattern of atrially induced closure of the mitral valve is present, it can be abolished by vagal stimulation. (Fig. 9, lower.) When the pattern of atrially induced closure of the mitral valve is not present, it can be induced by increasing sympathetic activity. (Fig. 12, lower.)

Summary of Effects of Cardiac Autonomic Nerve Stimulation. The central nervous system has available direct efferent pathways to the heart over which it can, at any given heart rate, systematically regulate ventricular contraction by either of two means. (1) It can control atrial contraction over a wide range, augmenting atrial

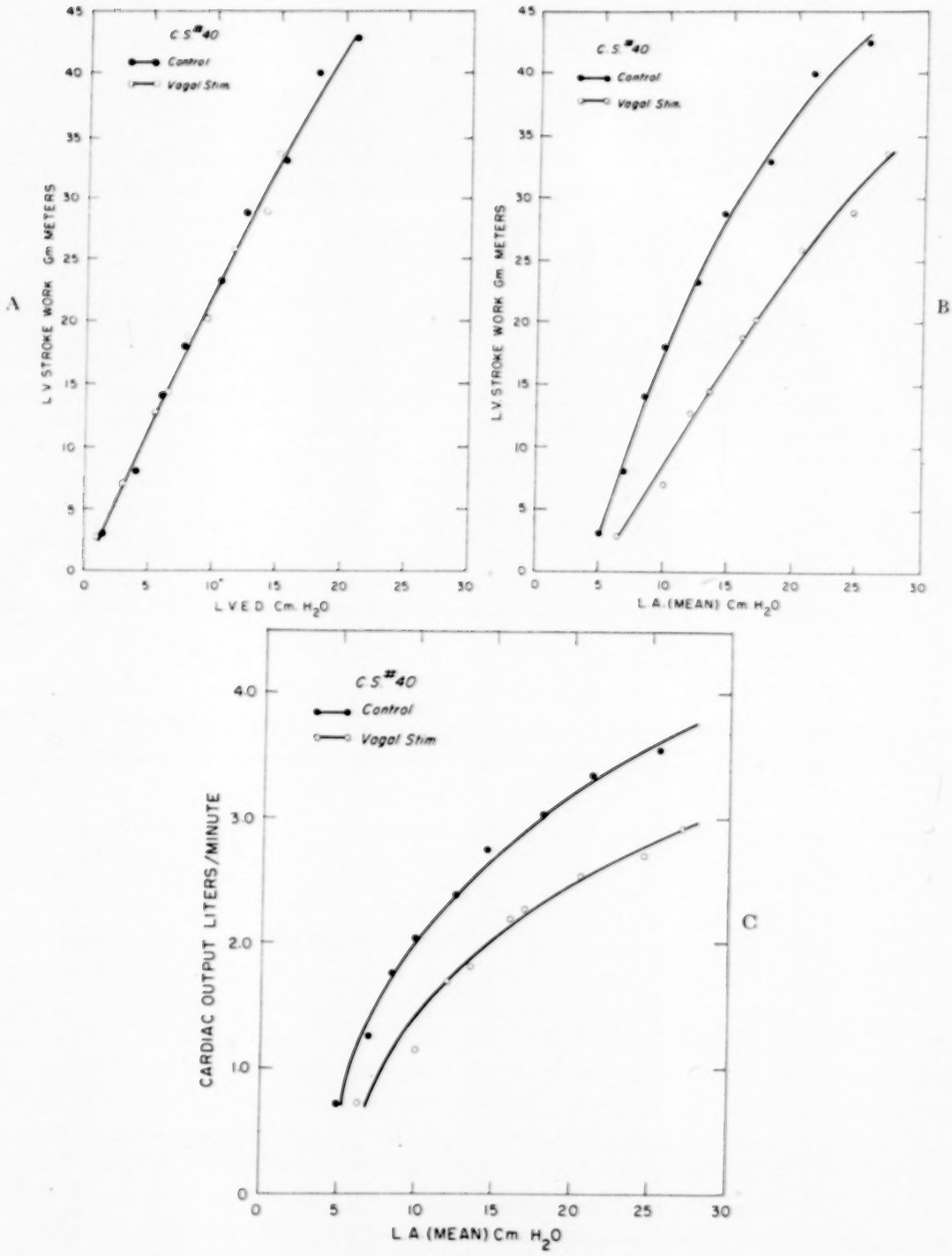


FIG. 8. A, relation of left ventricular end diastolic pressure and left ventricular stroke work before (solid circles) and during (open circles) stimulation of the distal cut end of the left vagus nerve (6.7 volts, 15 per second). Heart rate held constant at 187 per minute by left atrial pacing. Bilateral vagotomy. B, relation of mean left atrial pressure to left ventricular stroke work before and during vagal stimulation. C, relation of mean left atrial pressure to cardiac output before and during vagal stimulation. Data from all three panels are from the same experimental procedure.

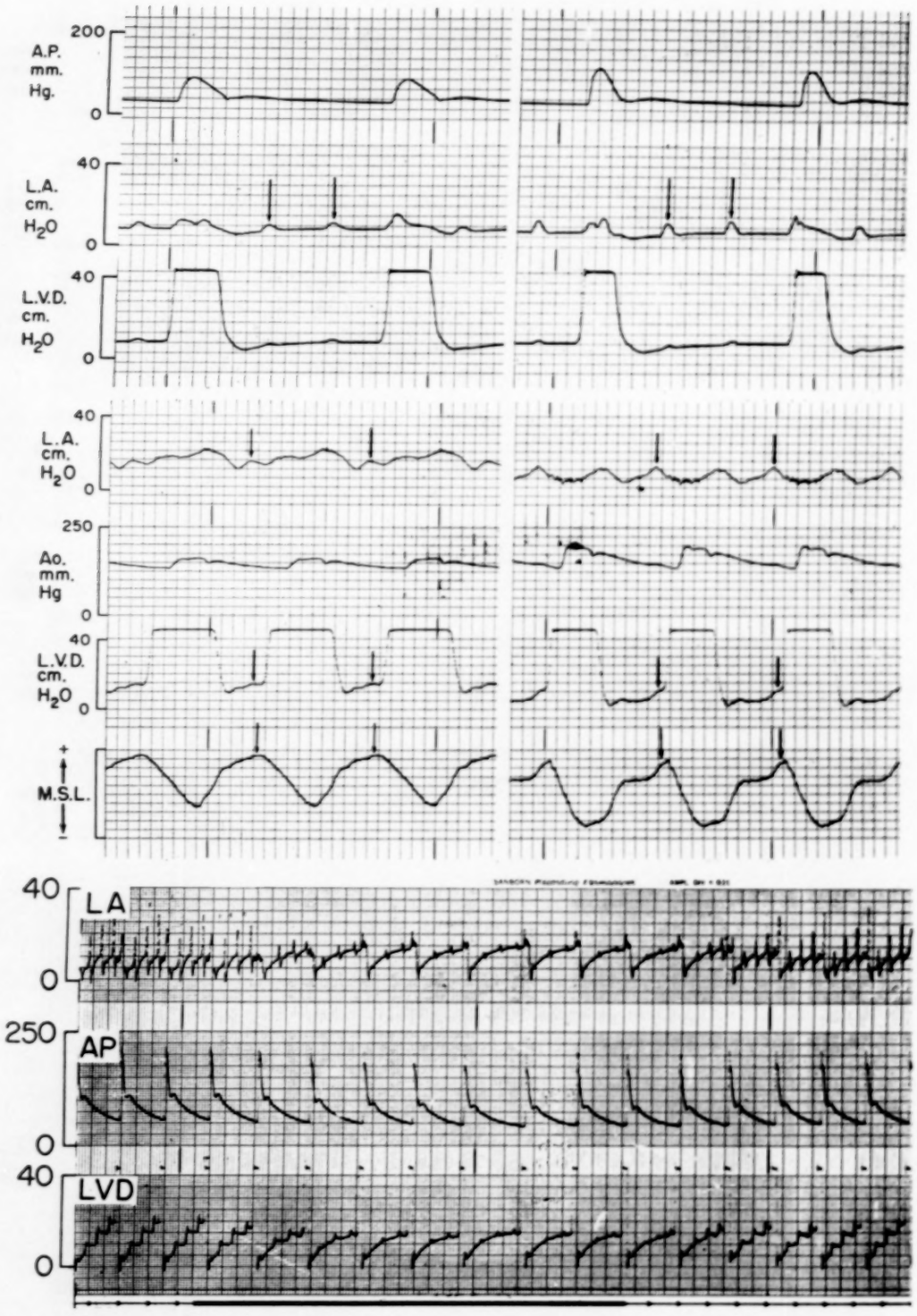


FIG. 9. *Upper*, surgically induced heart block, bilateral cervical vagotomy. Atrial rate held constant at 116 per minute by pacing. A.P. = aortic pressure; L.A. = left atrial pressure; L.V.D. = left ventricular diastolic pressure. Control tracing at left. Tracing at right taken during left stellate stimulation. Arrows indicate left atrial "a" wave during diastole. Chart speed = 100 mm. per second. *Middle*, heart rate held constant at 116 per minute by atrial pacing. Bilateral cervical vagotomy. Continuous stimulation of distal cut end of vagus nerve throughout experiment. Control tracing at left. Tracing at right taken during stimulation of the isolated left stellate ganglion. In each tracing the arrows

contraction by sympathetic stimulation and diminishing it by vagal stimulation. The ventricle is thereby presented with more or less blood at the end of diastole, its end diastolic pressure and fiber length are modified, and a consequent alteration is made in the vigor of its contraction. This can transpire in the absence of any change in the contractile characteristics of the ventricle. (2) The central nervous system, by way of cardiac sympathetic efferents, can directly cause the ventricle to contract more or less forcefully from whatever end diastolic pressure and fiber length has been obtained. The magnitude of the observed changes is noteworthy.

A more precise appreciation of the net effect of sympathetic impulses on the heart beating at any given rate may be stated as follows: The more forceful ventricular contraction resulting from sympathetic stimulation produces more complete systolic emptying, and consequently a lower diastolic impedance to ventricular inflow, i.e., the more complete systolic emptying places the ventricle on a more sensitive portion of its ventricular pressure-length and pressure-volume curve. It is in this circumstance, in which even a small increase in pressure produces a larger fiber length increase, that a more vigorous atrial systole propels blood and elevates ventricular end diastolic pressure.

It remains to consider the altered timing of events which accompanies these phenomena. Shortening the duration of each component of cardiac activity during sympathetic stimulation resulted in a longer ventricular diastole, thus allowing both a longer period for inflow and a longer interval during which ventricular relaxation can become complete. The cardiac sympathetic nerves may thus be construed, in an important sense, to be the guardian of diastole.

The shorter the diastolic interval, the more important it is for blood to enter the ventricle at an increased rate. Thus, not only does the contribution of atrial systole become most important at high heart rates, but the extent to which the atrial systole becomes shorter as well as more forceful will also help to determine the extent to which it can contribute to ventricular filling under these circumstances.

The alteration of the mechanical events of the cardiac cycle consequent to sympathetic stimulation correlates well with observed electrical phenomena; that is, the increased conduction velocity observed in the atrium, at the atrioventricular node, and in the ventricle [*42,43*]. There is also a shortening of the total refractory period and, with administered catecholamines, an increased excitability [*42*]. Since increasing the synchronicity of ventricular contraction results in production of more stroke work and stroke power from any given LVED pressure [*6*], it would appear unwise to attribute the increased ventricular work and power produced under sympathetic stimulation solely to a direct effect of the catecholamines on the myocardial fibers without making allowance for the obvious increase in the synchronicity with which they contract. This aspect of ventricular performance is construed to be a matter of importance [*4,6*].

The net effect of efferent vagal impulses, at least with the intensities of vagal stimulation used in these experiments, was to diminish the vigor of atrial contraction; they did not directly modify ventricular contractility.

THE NERVOUS CONTROL OF THE FRANK-STARLING MECHANISM: THE PRINCIPLES OF THE INNERVATED HEART

As a formal means of broadening the basic Frank-Starling relationship and of integrating it with the activity of the central nervous system in relation to acutely induced changes, two concise statements now appear to be appropriate for the heart operating at any given rate and aortic pressure, and in the absence of abnormal conditions such as hypoxia and acidosis.

(1) *If the effective catecholamine stimulus remains constant, the contraction of the ventricle varies directionally with its end diastolic pressure and fiber length; if the end diastolic pressure and fiber length remain constant, the contraction of the ventricle varies directionally with the effective catecholamine stimulus.*

(2) *The central nervous system has direct neural connections to the heart by means of which it can vary the left ventricular end diastolic pressure and fiber length while keeping the effective catecholamine stimulus constant (atrial systole), means by which it can vary the effective catecholamine stimulus, or both.*

indicate the left atrial "a" wave, the atrially induced increase in left ventricular end diastolic pressure and myocardial segment length. *Lower*, surgically induced heart block and bilateral cervical vagotomy. LA = left atrial and LVD = left ventricular diastolic pressure in cm. H_2O; AP = aortic pressure in mm. Hg. Stimulation of distal cut end of left vagus nerve during signal at bottom. Chart speed = 10 mm. per second.

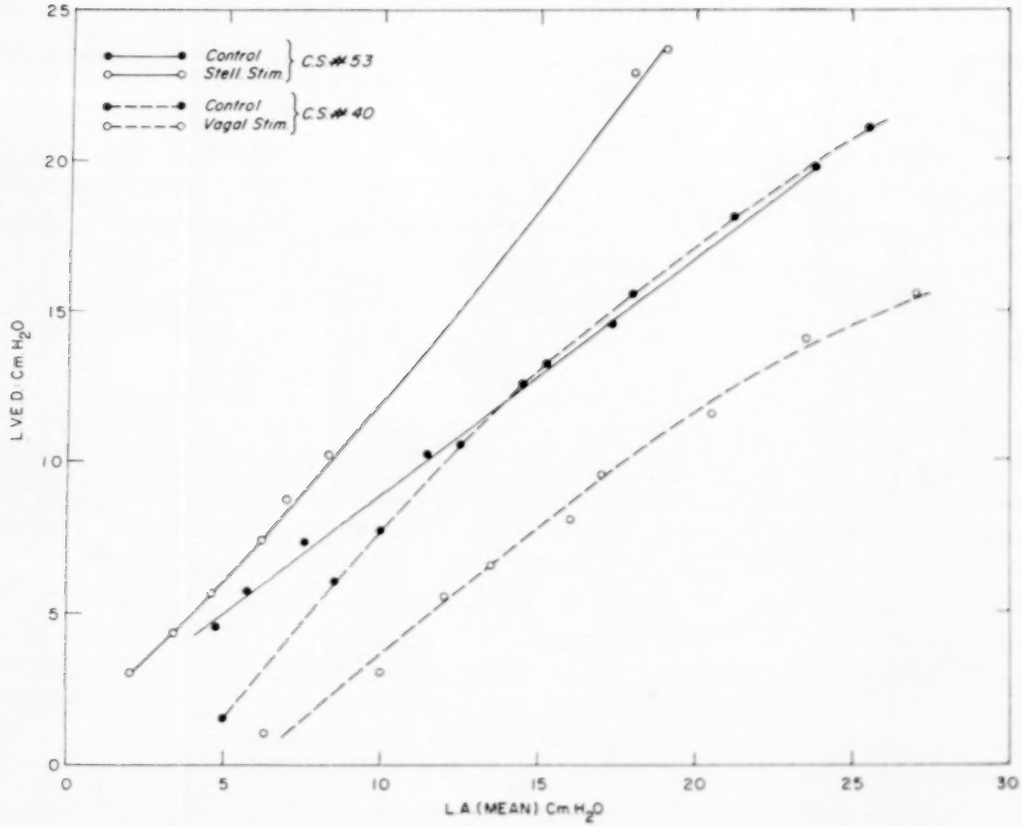
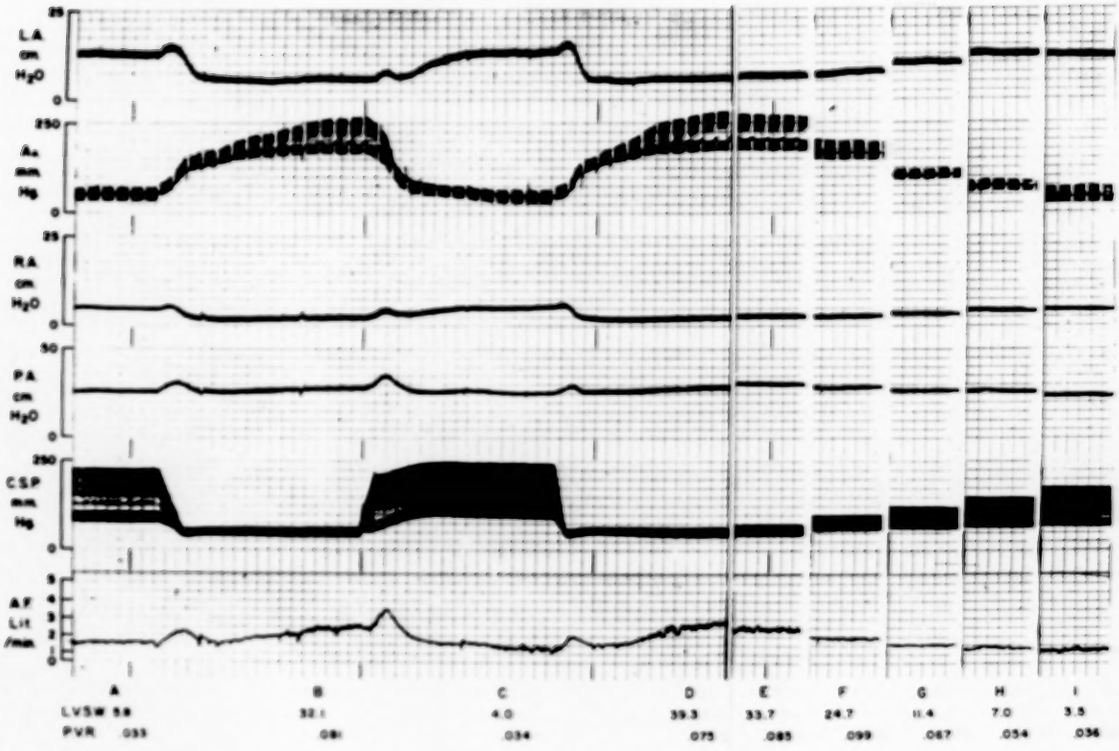


FIG. 10. L.V.E.D. = left ventricular end diastolic pressure; L.A., mean = mean left atrial pressure. Thecontinuous line joining solid circles shows the relation between mean left atrial pressure and left ventricular end diastolic pressure during a control run in which both pressures and the stroke volume were intermittently increased by the infusion of blood. Continuous line joining open circles shows the effect of left stellate ganglion stimulation on this relation. The dashed line joining solid circles shows the same relation in another experiment and the dashed lines joining the open circles shows the effect of efferent vagal nerve stimulation on this relation. Heart rate was held constant in both experiments by left atrial pacing.

THE INFLUENCE OF THE CAROTID SINUS ON THE PERFORMANCE CHARACTERISTICS OF THE HEART

The conventional view of the reflex function of the carotid sinus has been that it primarily influences heart rate and peripheral arteriolar resistance [*44*]. That the carotid sinus can influence the pressure-volume relation of veins has also been established [*45–49*]. More recently, attention has been focused on the changes in the contractility of the ventricle and atrium which can be reflexly induced by stimulation of the carotid sinus [*5*].

Carotido-Ventricular Reflex. Using a vagotomized preparation in which carotid artery pressure can be varied independently of systemic pressure [*5*], an examination was made of the effects of changing carotid artery pressure on myocardial contractility while heart rate is held constant. In the tracing shown in the upper left portion of Figure 11 the carotid perfusion pressure (C.S.P.) is changed. Initially, when the carotid pressure was high and pulse pressure large, aortic flow, aortic pressure and left ventricular stroke work were low in the presence of an elevated MLAP. When carotid perfusion pressure was lowered, aortic flow and pressure and left ventricular work rose substantially while mean atrial pressures fell. It is noteworthy that the increase in left ventricular stroke work, even at a lower MLAP, was several times the observed increase in peripheral vascular resistance. This result is a reproducible one and, as shown in the segmented panel in the upper right portion of Figure 11 a gradable effect. That is, the relation between filling pressure and external stroke work can be reflexly manipulated in a



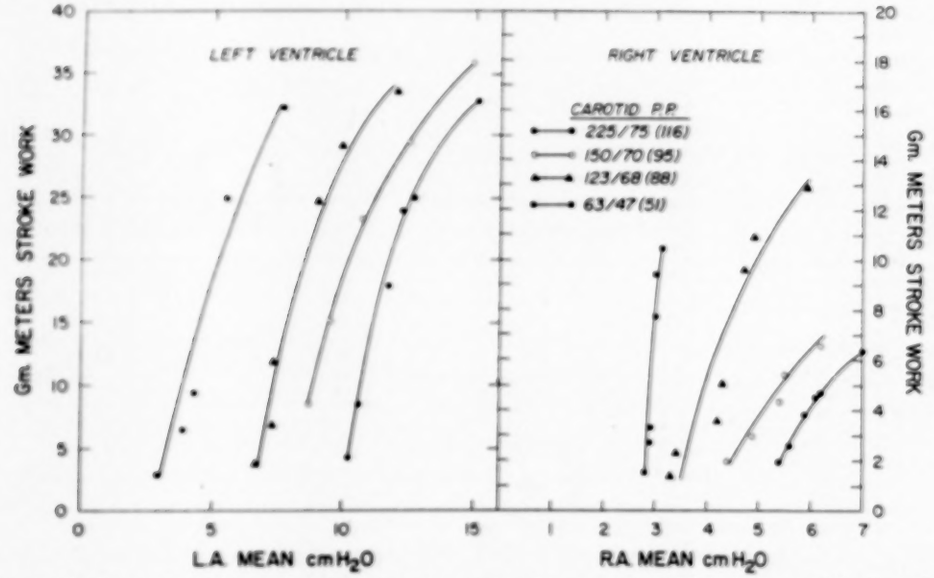


FIG. 11. *Upper*, L.A. = mean left atrial pressure; Ao. = aortic pressure; R.A. = mean right atrial pressure; P.A. = mean pulmonary artery pressure; C.S.P. = carotid artery pressure (generated by pump); A.F. = total aortic flow; L.V.S.W. = left ventricular stroke work; P.V.R. = calculated total peripheral vascular resistance (mm. Hg mean aortic pressure divided by aortic flow in milliliter per minute). Bilateral cervical vagotomy. Stellates intact. Heart paced at 200 per minute by atrial excitation. Chart speed = 0.5 mm. per second. Panel to the left (A, B, C and D) shows the hemodynamic effects of acutely changing carotid pressure. The changes in aortic flow which occur in the first thirty seconds after the pump induced change in carotid pressure are not reliable. The right panel shows the effects of graded changes in carotid pressure (E, F, G, H, I). Approximately two minutes intervene between each tracing segment. *Lower*, curves showing the effect of varying carotid pressure on the relation between mean left atrial pressure and left ventricular stroke work (left); simultaneously obtained curves relating mean right atrial pressure and right ventricular stroke work are at the right. The carotid pressures and the pertinent symbols are shown at the top right.

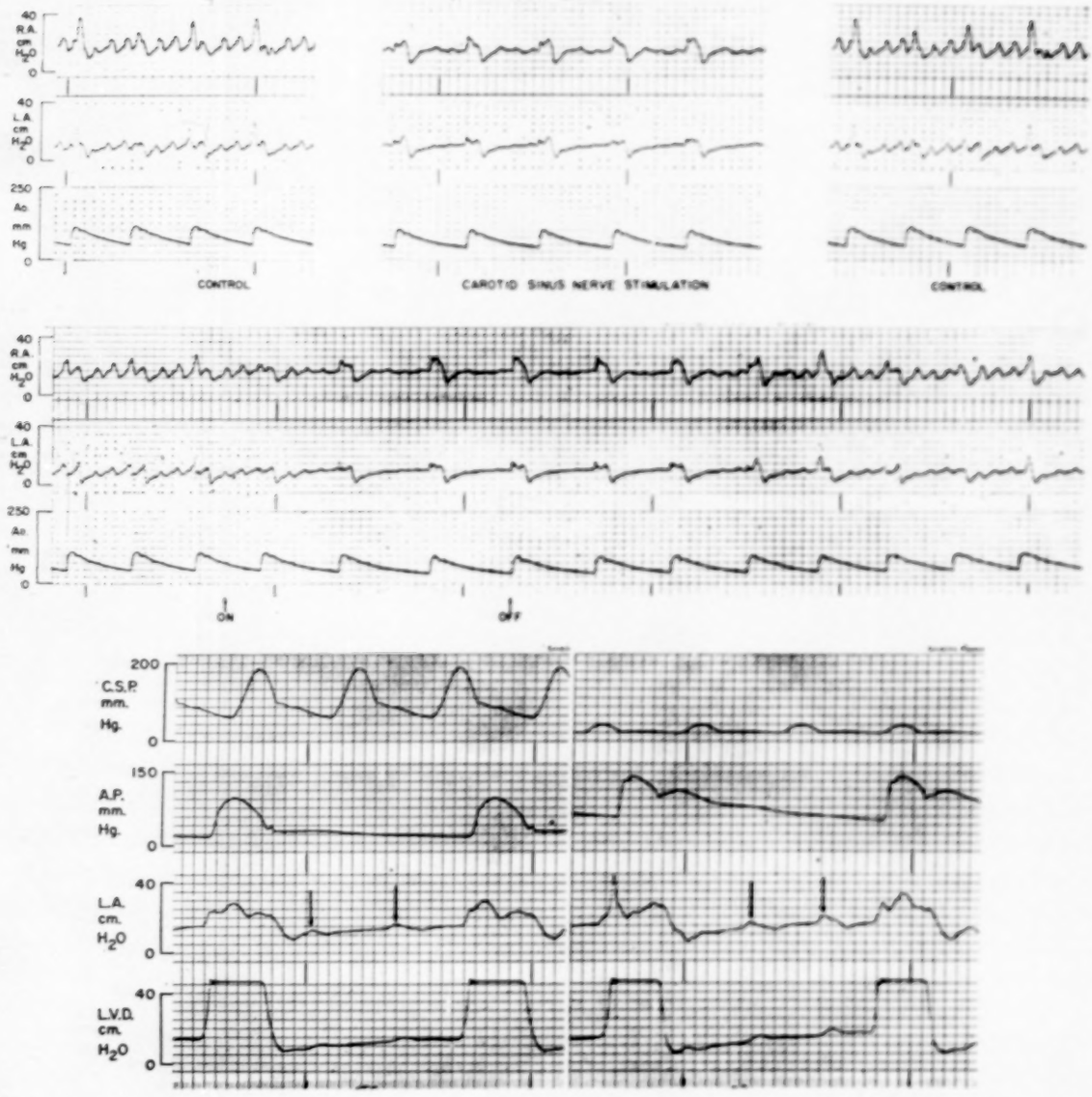


FIG. 12. *Upper*, R.A. = right atrial pressure; L.A. = left atrial pressure; Ao. = aortic pressure; Bilateral thoracic sympathectomy from stellate ganglia through T-5. Vagi intact. Surgically induced heart block; two or three atrial "a" waves appear in the interval between each ventricular beat. Atrial stimulation at rate of 167 per minute maintained throughout the entire experiment. Chart speed = 25 mm. per second. Segmented panel at left = control tracing. Middle segmented panel shows the "a" wave depression during carotid sinus nerve stimulation (6 volts, 10 per second and 4 msec.). Right segmented panel is again a control tracing. The long middle tracing shows rapidity with which reflexly induced depression of atrial systole begins. *Lower*, C.S.P. = carotid sinus pressure; A.P. = aortic pressure; L.A. = left atrial pressure; and L.V.D. = left ventricular diastolic pressure. Surgically induced heart block. The atrial "a" wave (arrows) during high carotid sinus pressure is shown in the left panel and during low carotid sinus pressure in the right panel. Chart speed = 100 mm. per second.

step-wise manner by making graded changes in the carotid perfusion pressure in much the same way that comparable changes can be induced by grading the frequency of left stellate stimulation.

By using the type of sequence shown in the upper right portion of Figure 11 the effect of varying the carotid perfusion pressure on the position of the ventricular function curve (VFC_{LA}) was observed. The lower portion of Figure 11 shows that when the carotid pressure is high, the VFC_{LA} is shifted to the right; when carotid pressure is low, the VFC_{LA} is shifted to

the left. At intermediate carotid pressures, the VFC_{LA} is situated correspondingly. Once again, the magnitude of the observed changes in stroke work at any given mean atrial pressure is of interest. The observed effects of carotid stimulation on the heart are diminished or abolished by sectioning stellate ganglion rami or by ganglionic blockade [5].

When carotid pressure is varied in such a vagotomized preparation, changes in MLAP of the type shown are accompanied by comparable changes in LVEDP, just as when direct sympathetic stimulation is applied [5].

It might be argued that the increase in ventricular contractility observed when carotid pressure is lowered can be attributed to the higher aortic pressure acting through the mechanism of homeometric autoregulation (p. 750). However, it has recently been determined that a marked increase in ventricular contractility is also observed when carotid hypotension is induced and aortic pressure as well as heart rate is kept at the same level [31].

Carotido-Atrial Reflexes. The carotido-vago-atrial reflex: In experiments on this reflex the sympathetic chain from the stellate ganglion down through T-5 is removed intact on both sides. This is performed so that any change in the atrial "a" wave which occurs can be attributed to reflex efferent vagal activity provided of course that the observed changes are within one circulation time. The upper portion of Figure 12 illustrates the depressant effect of carotid sinus nerve stimulation on the "a" wave of the paced atrium in a dog with surgically induced heart block, and the rapidity of the onset and wearing off of this effect. That vagal efferent fibers are responsible for this effect was shown by blocking the response with atropine [5].

The carotido-sympatho-atrial reflex: In experiments on this reflex a bilateral cervical vagotomy is performed so that the effects of changing carotid pressure or of carotid sinus nerve stimulation on the contraction of the atrium can be attributed to a sympathetic efferent pathway. A dog with surgically induced heart block is used. The lower portion of Figure 12 shows the effect of changing carotid pressure on the "a" wave of the paced atrium and the reflected effect thereof on left ventricular diastolic pressure.

Function of the Carotid Sinus. A pattern of the baroceptor's functional role has been evolved which brings together a variety of observations in a manner that seems to have an appealing unity. This position holds *that a dominant physiologic responsibility of the carotid sinus in circulatory regulation is to augment or diminish the contraction of the ventricle.* The basis for this is as follows:

(1) Carotid hypotension diminishes venous distensibility. The net effect of such a change, if it alone occurs, is an increased ventricular end diastolic pressure and fiber length, and thus an augmented ventricular contraction. Splenic contraction has the same effect.

(2) Carotid hypotension augments and shortens the atrial contraction by means of the carotido-vago-atrial and the carotido-sympatho-atrial reflexes. The net effect of such an atrial augmentation, if it alone occurs, is an increased ventricular end diastolic pressure and fiber length, and thus an augmented ventricular contraction.

(3) At any given heart rate carotid hypotension directly augments the stroke work and stroke power produced by the ventricle from any given end diastolic pressure or fiber length.

(4) Since carotid hypotension directly augments ventricular stroke power by shortening the systolic time for any given amount of work produced, and also produces a more rapid rate of relaxation, it thus provides for a longer interval of diastolic filling than would otherwise occur. This factor becomes especially important when heart rates are high.

(5) The more complete systolic emptying consequent to carotid hypotension places the ventricle on a lower and more sensitive portion of its diastolic pressure-length curve. As a result there will be more filling and a greater fiber length elongation produced by any given atrial systole than if more complete systolic emptying had not taken place.

(6) The increased peripheral vascular resistance during carotid hypotension maintains a higher aortic pressure at any given stroke volume and heart rate than would otherwise be present. In addition to maintaining an adequate pressure for coronary perfusion, the higher aortic pressure produces an increased ventricular contractility through homeometric autoregulation.

(7) Tachycardia *per se* increases ventricular contractility through homeometric autoregulation in addition to the concomitant inotropic influence of the increased sympathetic outflow.

(8) Whatever catecholamines are secreted by the adrenal medulla in response to a lowering of carotid sinus pressure would be expected to re-enforce the effects enumerated.

The intended purpose of synthesizing the available information in this manner is not to denigrate the importance of changes in heart rate *per se* or to minimize the importance of regional changes in peripheral vascular resistance but rather to invite re-evaluation of the proper role of the carotid sinus in circulatory regulation. It seems fair to insist that the view of the carotid sinus as a sense organ which acts primarily to safeguard blood flow to the vital organs, such as the brain and heart, is no longer a tenable position. It would seem much more appropriate to cast it in the role of a sensing element which helps to regulate blood flow to all tissues of the organism in accordance with their activity and metabolic requirements [*50–52*]. To a substantial extent the baroceptor operates much like a voltage regulating element in an electrical system; i.e., it causes an appropriate variation of input so as to maintain a constant voltage when the current requirements of the system it is supplying are changed. An example of its operation in this manner was obtained recently in experiments in which it was demonstrated that local muscular activity effectively produces a functional sympathectomy in the vascular bed of the active area [*53*]; under such circumstances it can be shown that when carotid pressure is elevated, thus inhibiting the stimulatory action of its reflex autonomic activity, blood flow through the active muscular area is lower, venous pO_2 and pH from it are decreased, and arteriovenous O_2 difference across it is widened, each by substantial amounts relative to what these values are when carotid pressure is lower [*53*].

The Interrelation of Intrinsic Mechanisms and Extrinsic Influences. It is clear that the left ventricle of the isolated heart exhibits increased contractility through homeometric autoregulation when its activity is increased simply by increasing the aortic pressure. (Fig. 2.) It is also clear that, starting at any given level of aortic pressure, sympathetic stimulation increases contractility in advance of and independently of an increased aortic pressure [*4*]. Both must, therefore, be playing a role in producing the observed increase in ventricular contractility induced by carotid hypotension. It seems peculiarly appropriate to the operation of the carotid sinus that its cardiac and peripheral vascular effects interrelate so as to reinforce each other, and further that an increase in the norepinephrine background resulting from sympathetic stimulation facilitates the intrinsic mechanism of homeometric autoregulation.

It is now apparent that the Bowditch staircase effect is operative in the adequately supported canine heart and thus that an increase in rate will, of itself, either increase contractility or protect against the extent to which contractility might otherwise diminish. It is also clear that, at any given heart rate, sympathetic stimulation increases contractility. Both must therefore play a role in producing the observed increase in contractility when carotid pressure is lowered in the normal organism. Again, it is appropriate for the operation of the carotid sinus that these effects are reinforcing rather than opposing.

Since the carotid sinus can, both directly and indirectly, reflexly modify both the filling and the contractility of the ventricles by such diverse means and over such wide ranges, it should be considered to play a role as one significant influence in the control of cardiac output in varying states.

THE OPERATION OF INTRINSIC MECHANISMS AND EXTRINSIC INFLUENCES IN THE INTACT ORGANISM

The pumping action of active muscles on the venous bed, reflexly induced venoconstriction, the extent to which the atria can so markedly vary the amount of blood they propel into the ventricles—on the basis of known mechanisms these achieve substantial physiologic significance only to the extent that they influence ventricular filling, stroke work and stroke volume, i.e., heterometric autoregulation. In addition, the continuing beat-to-beat operation of Starling's law of the heart, with both the right and left ventricles operating on their respective curves provides a convincing explanation (indeed, the only feasible explanation put forward thus far) of how the ratio between systemic and pulmonary blood volumes is constrained within such narrow limits for the lifetime of the organism. Unlike homeometric autoregulation, the beat which follows immediately after an increase of end diastolic pressure and fiber length produces more external work than the one immediately before it. As shown most convincingly by Brecher [*54*], there are substantial variations in venous return throughout the respiratory cycle. The variations of venous return to the right and left ventricle cannot be the same with respect to either amount or time; in this regard the ven-

tricles are, in fact, often reciprocating with each other to varying degrees. Lastly, the importance of changes in left ventricular volume relative to its stroke work throughout the respiratory cycle, and also the fact that this relationship still obtains after changing the circulatory state by inducing exercise, has been firmly established [*55*].

Since the studies of Stead and Warren [*56*], significant work has been carried out on the question of whether or not the Frank-Starling mechanism is operative in normal man [*57,58*]. From the work of Braunwald et al. [*59,60*] there can no longer be any doubt that the ventricular fiber of the heart enclosed in the thorax of unanesthetized man will contract more forcefully from a longer initial length. The observation that the heart may not enlarge or may even become smaller during exercise is hardly an indication that heterometric autoregulation is no longer operative; if this were true the lungs would become either dry or inundated in a short period of time. What it does indicate is that the central nervous system has increased myocardial contractility and thus shifted the ventricular function curve to the left as a result of sympathetic stimulation since tachycardia has occurred; homeometric autoregulation has undoubtedly also taken place. The principles of the innervated heart stated herein, considered in conjunction with the observations on homeometric autoregulation, appear to embrace the observed phenomena in a reasonable manner at the present time. As ever, it is to be hoped that new findings and more sophisticated technics of analysis may in the future yield a more widely applicable and more satisfactory simplification of the facts.

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Clinical Studies

The Mechanism of Particulate Carrier Reactions*

III. The Stabilizing Effect of Serum Proteins

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PARTICULATE carrier agglutination reactions have been widely employed as simple yet highly sensitive procedures for the detection of small amounts of antibody. The procedures employed consist, in general, of coating the carrier particles with a suitable antigen, and using these coated particles to detect antibody in serums or other fluids. The presence of various protein or carbohydrate components in the material being tested may result, however, in prozone effects, titer reductions, or absence of agglutination. These effects have usually been ascribed to the presence of "inhibitors" or "stabilizers" in the test sample; frequently these two terms have been used interchangeably and without distinction. The presence of serum protein components in particulate carrier reactions, for example, will modify the surface properties of the particles and thereby influence the reaction. This may be due to blocking of specific reactive sites or to changes in the stability of the particle as a result of non-specific adsorption of these components to the particle surface.

Serological tests for rheumatoid arthritis involve a reaction between rheumatoid factors (RF) and gamma globulin. If gamma globulin is adsorbed to the surface of particulate carriers, such as red cells, latex or bentonite particles, reaction with RF results in agglutination or flocculation of the particulate carriers. If gamma globulin is added previously to the serum of a subject with rheumatoid arthritis, the RF is neutralized, and is no longer reactive with sensitized particulate carriers. This constitutes an inhibition reaction.

In all particulate carrier systems, rheumatoid arthritis serum reacts only with gamma globulin and not with any other plasma proteins. The objective of this investigation was to demonstrate that plasma proteins other than gamma globulin do not act as inhibitors but exert their primary effect as stabilizers of particulate carriers, due to their colloidal protective effects.

MATERIALS AND METHODS

(1) Protein preparations: Fii Squibb (No. 1,812); Fiv Pentex and Fv Pentex were used. Fv was purified by precipitation with half-saturated ammonium sulphate solution. The resulting precipitate was redissolved in physiological saline solution and dialyzed against several changes of saline solution. Paper electrophoresis revealed only albumin, with no trace of gamma globulin. The Fiv preparation employed also was free of both albumin or gamma globulin. It consisted of 30 per cent α_1 globulin, 65 per cent α_2 globulin and 5 per cent β globulin.

(2) Latex particles: Dow polystyrene latex particles‡ 0.81 μ diameter in a suspension containing 27.6 per cent solids.

(3) Purified rheumatoid factor:§ a 19S macroglobulin, 1 μg. N/ml., was prepared by the method of Heimer et al. [1] from the serum of a patient with rheumatoid arthritis. This preparation was free of

‡ Courtesy of Dr. J. Vanderhoff, Dow Chemical Co., Midland, Michigan.

§ Courtesy of Dr. R. Heimer, Hospital for Special Surgery, New York, New York.

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7S gamma globulin and gave high titers with the FII latex particle (FII L.P.), FII coated tanned sheep cell (FII S.C.), heterophil absorbed sheep cells (S.S.C.), and sensitized human D-erythrocyte (S.H.C.-D.) tests [5].

(4) Serum No. 2,346 was collected from a patient with rheumatoid arthritis.

(5) Rabbit anti-human albumin serum (RAHA):* In order to remove anti gamma globulin activity from the RAHA without introducing extraneous gamma globulin, the following procedure was used: The RAHA was inactivated at 56°C. for thirty minutes. One volume of packed tannic acid-treated sheep erythrocytes sensitized with human gamma globulin (HGG) was added to two volumes of a fivefold dilution of RAHA. The mixture was allowed to stand one hour at room temperature and then overnight in the refrigerator. After centrifugation the supernatant was examined for the presence of agglutinins with the FII S.C. Test [3].

(6) All experiments were performed in glycine saline buffer with a pH of 8.2 [6].

(7) Tannic acid (Mallinckrodt).

(8) Nitrogen contents were determined by the method of Lowry et al. [7].

(9) Test procedures: FII L.P. according to the method of Singer and Plotz [2,12]; FII S.C. by the method of Heller et al. [3].

RESULTS

The experiments listed in Table I were performed to determine the effect of Fv and mixtures of Fv and FII on the stability and agglutinability of latex particles. Varying amounts of Fv-FII mixture were added to 5 ml. of a latex suspension (1.21 mg. solids/ml.), and the final volume was adjusted to 10 ml., with glycine saline buffer (pH 8.2). The samples were allowed to stand at room temperature for thirty minutes and then centrifuged for thirty minutes at 12,000 revolutions per minute. The supernatant was discarded and the sediment resuspended in 10 ml. of buffer. The washing procedure was repeated twice in order to remove excess unadsorbed protein. The final volume was adjusted to 5 ml. After twenty-four hours, the samples were centrifuged at 2,000 revolutions per minute for fifteen minutes. Aliquots (0.1 ml.) of the supernatants were diluted one hundred times, and their optical densities read in a Coleman Universal Spectrophotometer Model 11 at 650 mμ. Latex particles treated only with FII were completely precipitated, whereas latex particles treated with Fv remained stable.

* Courtesy of Dr. R. Heimer, Hospital for Special Surgery, New York, New York.

TABLE I

AGGLUTINATION TITERS OF LATEX PARTICLES SENSITIZED WITH MIXTURES OF FRACTIONS II AND V*

Total Amount of Protein Added to 10 ml. Final Volume		Supernatant Optical Density	Reciprocal of Agglutination Titers		
FII (μg. N)	Fv (μg. N)		Anti-albumin	Rheumatoid Factor	Serum No. 2,364
700	0	0.010	0	25,600	12,800
700	400	0.014	...	...	...
700	600	0.036	6,400	25,600	6,400
700	800	0.046	...	...	...
700	1,000	0.149	6,400	12,800	3,200
700	1,400	0.260	6,400	3,200	1,600
700	1,600	0.310	...	...	...
700	2,000	1.16	6,400	800	1,600
0	700	1.31	6,400	0	0
0	0	...	0	0	12,800

* Protein excess removed by washing particles.

(Table I.) Latex particles treated with FII-Fv mixtures showed increasing stability as increasing amounts of Fv were added.

The sediments were then resuspended in their own supernatants by shaking. Even the samples which were completely precipitated could be redispersed. These suspensions were then diluted one hundred times with glycine buffer (pH 8.2). Two progressive serial dilutions in 0.5 ml. volumes of RAHA, purified RF or serum No. 2,364 were added to 0.5 ml. amounts of each coated latex suspension. Titers were determined according to the standard FII LP procedure. The diluted suspensions were stable as evidenced by absence of agglutination in control tubes. Titers are given in Table I.

These results indicate that latex particles can be coated by Fv or FII or both in mixtures. Adsorption of FII to latex particles has previously been demonstrated [8]. The antialbumin titers are unaffected by the relative amount of Fv in the coating mixture. On the other hand, the RF titers are markedly decreased by increasing amounts of Fv in the coating mixture.

This difference in reactivity may be due to the difference in the specificity of the two reactions. Thus the albumin-antialbumin reaction is not influenced by the stabilizing effect of increasing Fv. The relative avidity of RF for FII on the latex particle is much less, and is there-

TABLE II
RHEUMATOID FACTOR AGGLUTINATION TITERS USING LATEX PARTICLES SENSITIZED WITH FII-FIV AND FII-FV MIXTURES*

Total Amount of Protein Added to 10.0 ml. Final Volume		Reciprocal of RF Agglutination Titer	
FII (μg. N)	FIV or FV (μg. N)	FII-FIV	FII-FV
0	200	0	0
20	180	800	3,200
50	150	1,600	3,200
100	100	6,400	25,600
150	50	6,400	25,600
180	20	6,400	51,200
200	0	51,200	51,200

* Latex particles not washed.

TABLE III
RHEUMATOID FACTOR AGGLUTINATION TITERS USING LATEX PARTICLES SENSITIZED BY SEQUENTIAL TREATMENT WITH FII AND FV OR FV*

Total Amount FIV or FV (μg. N) Added to 10 ml. Final Volume	Reciprocal of Agglutination Titers			
	FIV Added First	FIV Added Second	FV Added First	FV Added Second
0	51,200	25,600	51,200	51,200
1	12,800	25,600	51,200	51,200
10	6,400	51,200	25,600	25,600
100	1,600	25,600	12,800	12,800
500	0	6,400	6,400	6,400
1,000	0	1,600	3,200	3,200

* Constant amount 100 μg. N FII used. Latex particles not washed.

fore markedly affected by Fv stabilization. The stabililizing effect of Fv counteracts the tendency of RF to agglutinate the particles.

The fact that identical antialbumin titers were obtained with all coating mixtures is consistent with previous observations that RF agglutination titers are unaffected by sensitizing latex particles with increasing amounts of FII and subsequent removal of excess unadsorbed proteins [6].

Uncoated latex particles were agglutinated in high titer by rheumatoid arthritis serum (Table I), but not by purified RF or RAHA. In order to avoid effects due to unknown serum components, subsequent experiments were performed only with purified RF.

The results of experiments with FII-FIV and FII-FV mixtures are recorded in Table II. One-tenth milliliter of concentrated latex suspension (1.21 mg./ml.) was added to 8 ml. glycine buffer (pH 8.2). Sufficient buffer and protein mixture were then added to yield a final volume of 10 ml. and a protein concentration of 20 μg. N/ml. These amounts were sufficient to achieve optimal sensitization without leaving a significant excess of protein in solution. Consequently, washing could be omitted. Titers against purified RF were recorded. Increasing amounts of either FIV or Fv resulted in lower titers. FIV was much more effective in this respect than Fv. The reductions in titers were not due to the decreased proportion of HGG in the coating mixture since it has been shown that optimal sensitization can be achieved with as little as 1 μg. N FII/ml. [6]. Furthermore, amounts of FII up to 20 μg. N/ml. will not inhibit the reaction between RF and latex particles. Therefore, the decreased titers in Table II can be due only to the presence of either FIV or Fv. These results parallel the data in Table I.

In Table III are shown the results when the protein fractions were added sequentially to the latex particles. In all cases a constant amount of 100 μg. N FII was employed for sensitization. The addition of FIV first, before sensitization with FII, was more effective in the reduction of titers than when added after sensitization with FII. The difference in effect of FIV as compared to Fv parallels the results given in Table II.

It may be estimated from previous data on FII adsorption [8] that 10 μg. N FII is sufficient to cover completely the surface of the latex particles in the 10 ml. suspension. If the particles are first treated with FIV (500 to 1,000 μg. N), apparently little or no FII is adsorbed, as indicated by negative titers at these levels. In the case of Fv, FII adsorption apparently can still occur, possibly by displacement of Fv molecules. When FII is added first, displacement by Fv or FIV is unlikely [9]. However, FIV or Fv are smaller molecules and their adsorption in "holes" in the FII surface is not excluded.

Table IV summarizes results obtained with the FII S.C. procedure. One volume of tanned sheep cells was exposed to two volumes of FII, incubated at 37°C. for thirty minutes, washed free of exposed protein, and then exposed to a same

TABLE IV
AGGLUTINATION TITERS USING TANNED SHEEP CELLS SENSITIZED WITH FII-FIV AND FII-FV SEQUENTIALLY AND IN MIXTURES

Order of Addition of Proteins	Reciprocal of Agglutination Titers	
	Rheumatoid Factor	Antialbumin
FII only	25,600	0
FII first, FV second	25,600	25,600
FV first, FII second	1,600	25,600
FII and FV mixture	1,600	51,200
FV only	0	51,200
FII first, FIV second	51,200	...
FIV first, FII second	1,600	...
FII and FIV mixture	1,600	...
FIV only	0	...

amount of Fv. In a second experiment the order of protein treatment was reversed. The agglutinability of cells treated this way was compared with that of tanned cells coated by equal mixtures of FII and Fv and to cells coated by each alone. All protein concentrations were 600 μg. N/ml.

Tanned cells coated with FII were agglutinated by RF, while cells coated with FIV were agglutinated by RAHA. Coating with a mixture of the two proteins yielded cells which could be agglutinated by both test systems. These results indicated that tanned sheep cells can be coated by either protein or by both in a mixture. The presence of Fv in the coating mixture sharply reduced the RF titer. On the other hand, FII in the mixture did not affect the antialbumin titer. The order of sequential coating was of little significance with regard to antialbumin activity but was important for RF activity. Thus, coating first with Fv sharply reduced the RF titer, but when Fv was used as a second coating there was no change in RF titer. In sequential coating both proteins were adsorbed to the cells, as evidenced by activity with both test systems.

Tanned cells coated with Fv yielded RAHA titers eight times higher than similarly coated latex particles. Latex particles coated with a mixture of equal parts of FII and Fv showed no reduction in RF titer. (Tables I and II.) Tanned cells similarly coated exhibited much lower RF titers. Similar experiments using FIV are also listed in Table IV. The results parallel those obtained with Fv.

In Table V the effect of varying amounts of FIV on the agglutinability of latex particles or tanned sheep cells, using a constant amount of RF or rheumatoid serum is illustrated. FIV was chosen for this series because of its greater effect on RF titers than Fv. One-fourth milliliter aliquots of FIV, varying from 0 to 500 μg. N, were mixed with 0.25 ml. of a 1:500 dilution of RF or serum No. 2,346. After ten minutes incubation at 37°C., 0.25 ml. of unsensitized latex particles, or latex particles sensitized with 10 μg. N/ml. FII or tanned coated sheep cells were added to each tube. The usual procedures for the FII L.P. and FII S.C. were carried out.

Both RF and serum No. 2,346 agglutinated sensitized latex particles or tanned sheep cells in the absence of added FIV. When sufficient FIV was added, agglutination of sensitized latex

TABLE V
THE INFLUENCE OF ADDED FIV ON THE AGGLUTINABILITY OF LATEX PARTICLES (LP) AND TANNED SHEEP CELLS (SC) BY THE RHEUMATOID FACTOR AND RHEUMATOID SERUM

Test System	Amount FIV added (μg. N/ml. test system)							
	500	250	125	62.5	31.2	15.6	7.8	0
Rheumatoid factor (1:500) unsensitized LP	−	−	−	−	−	−	−	−
Rheumatoid factor (1:500) FII LP	−	−	−	+	+++	+++	+++	+++
Rheumatoid factor (1:5000) FII SC	+++	+++	+++	+++	+++	+++	+++	+++
Serum No. 2,364 unsensitized LP	−	−	−	−	++	+++	+++	+++
Serum No. 2,364 FII LP	−	−	+	++	+++	+++	+++	+++
Serum No. 2,364 FII SC	+++	+++	+++	+++	+++	+++	+++	+++

particles was prevented. These experiments represent an artificially induced prozone effect due to large amounts of added Fiv. Tanned sheep cells showed no prozone effect.

As expected, unsensitized latex particles are agglutinated by a rheumatoid serum but not by purified RF. Agglutination by rheumatoid serum is suppressed by Fiv; however, only 62.5 μg. N were required to abolish agglutination as compared to 250 μg. N for sensitized latex particles. This is presumably due to the fact that Fiv is adsorbed with the unsensitized particles onto the particle surface at the same time as HGG and therefore interferes with complete coating by HGG.

COMMENTS

It has been established that the sensitivity of the S.S.C. and Fii L.P. tests can be increased by using the euglobulin fraction of the rheumatoid patient's serum [*10–14*]. This enhanced sensitivity has been attributed to the removal of inhibitors present in whole serum. Two types of inhibitors have been demonstrated: one associated with gamma globulin alone [*3,11*], the other with the remaining serum proteins [*14–16*]. Proteins such as gelatin and albumin [*15,16*] have been shown to reduce agglutination titers. This has been attributed to their protective colloidal action. The fact that whole serums from human subjects and animals do not sensitize latex particles is probably due to the same mechanism [*15*].

The role of serum proteins in the serologic reactivity of particulate carrier systems may be viewed in terms of their effect on the colloidal stability of carrier particles. Latex is a colloidal suspension of hydrophobic particles. The stability of the suspension is maintained through the presence of surface active agents [*17*]. Even so, the latex particles are in comparatively unstable suspension. Thus the addition of small amounts of protein (10 μg. protein per mg. latex) will cause latex particles to flocculate, whereas larger amounts increase the particle stability. This is the typical protective effect of a hydrophilic colloid on a hydrophobic one. As small amounts of the protein are adsorbed, the initial surface charge of the latex particle is neutralized, causing instability. Upon further addition of protein, the particles acquire a net charge and a surface due to the adsorbed protein and are thereby stabilized.

In contrast, erythrocytes have, in comparison, a much more hydrophilic surface, and erythrocyte stability is essentially unchanged by the addition of serum proteins. Thus small amounts of protein will agglutinate a latex suspension but not a red cell suspension.

The stabilizing effect of serum proteins on latex particles varies with the nature of the protein and with the pH of the system. At a pH of 8.2, for example, concentrated latex suspensions are destabilized by Fii but are stabilized by Fv. (Table i.) The stabilizing effect of Fv is essentially due to its more hydrophilic nature and to its greater net charge as compared to Fii at a pH of 8.2.

In the performance of the Fii L.P. test it has been observed that rheumatoid serums often exhibited a prozone phenomenon [*12,13,18–20*]. This prozone effect has been variously ascribed to a thermolabile substance with the characteristics of serum complement [*21,22*] or to an alpha$_2$-globulin inhibitor [*23*]. In the present study artificial prozones were induced in the Fii L.P. test with purified RF by the addition of Fiv. (Table v.) In part, at least, the prozone effect must be attributed to the non-specific role of high concentrations of serum proteins in the test systems. At these high concentrations the latex particles can adsorb the various serum proteins. The adsorbed proteins will stabilize the particles and counteract the agglutinating effect of RF. The absence of prozones in the tanned erythrocyte agglutination system (Table v) is consistent with this view, since erythrocyte stability is little influenced by added serum proteins.

In addition to the prozone effect, the presence of Fractions iv or v also causes titer reductions in the Fii L.P. system. This is the case whether the protein is added before or after sensitization (Table iii) or as a mixture with the sensitizing Fii. (Tables i and ii.)

Competition for the available particle surface by the various proteins may occur. When 500 μg. N Fiv was added prior to sensitization of latex particles with Fii, no agglutination was obtained. (Table iii.) This could be due to complete covering of the surface by Fiv, and subsequent addition of Fii was therefore ineffective. On the other hand, prior sensitization by Fii did not prevent titer reduction by Fiv or Fv. Of these three proteins, Fii has the largest molecular size. Even if the particle surface were completely saturated by Fii molecules, it would be expected (assuming Fii adsorption is a random process

and that the adsorbed protein film is immobile), that the surface would still have available holes large enough to accommodate the smaller FIV or FV molecules [*8,24*]. Consequently, latex particles already completely coated by FII could still adsorb other serum proteins and be stabilized by them.

With the FII S.C. system the results were similar except that treatment with FIV or FV after sensitization with FII was without effect. (Table IV.) This latter result confirms the view that serum proteins, even if adsorbed to the tanned cell, have little effect on their stability. Titer reduction by protein mixtures or by pretreatment with FIV or FV is probably due mainly to reduction in FII adsorption.

Franklin [*25*] has demonstrated that alpha$_2$-globulin prevents agglutination in the sensitized sheep cell test. In this procedure sensitization is effected by means of an immune reaction, whereas latex particles or tanned cells are sensitized by non-specific adsorption of FII. In the S.S.C. system only a small fraction of the surface is occupied by the immune globulin, and non-specific adsorption can still take place freely on the red cell surface [*26*]. Since the sheep cells are sensitized with an amount of immune globulin just below that capable of causing agglutination, it is apparent that they are much more likely to agglutinate than are tanned cells. Consequently, adsorbed serum proteins can in this case exert a protective effect.

It has been demonstrated that many different protein or polysaccharide antigens may be attached to sheep cells and that all are reactive with their respective antibodies [*27–29*]. Latex particles as well as sheep cells may be coated with two antigens, as evidenced by the fact that when they are coated with a mixture containing equal amounts of FII and FV, they exhibit titers with both RF and RAHA. RF titers with latex are not reduced by the presence of an equal amount of FV as compared to FII, but RF titers with tanned cells are sharply reduced.

It appears that FV is more strongly adsorbed to tanned cells than to latex particles and that the reverse is true for FII which is consistent with the more hydrophilic nature of albumin as compared to gamma globulin. Abramson [*9*] has shown that collodion particles adsorb globulins more strongly than albumin but that the reverse is true for quartz particles.

The term inhibitor has been widely used to characterize the effect of proteins or serologic reactions with rheumatoid factor, but this term is somewhat misleading. It seems likely that serum protein fractions other than FII do not act as inhibitors or rheumatoid factor. Rather, their primary effect in the latex system, and possibly in the sensitized sheep cell system, is that of stabilizers due to their protective colloidal action. The term inhibitor is appropriate only when the rheumatoid factor is neutralized, and sensitized carrier agglutination prevented, as is the case with aggregated gamma globulin.

SUMMARY

1. FII and FV alone or in mixtures are adsorbed onto the surface of latex particles or tanned sheep cells and produce agglutination with both rheumatoid factor and antialbumin systems.

2. Adding FV or FIV stabilizes latex particles and lowers rheumatoid factor titers by their protective colloidal action. FIV is more effective than FV in this respect.

3. Added FIV produces a prozone effect with the FII latex particle system but not with the FII sheep cell system.

4. The probable mechanism of the protective action of serum protein fractions on particulate carrier systems is discussed.

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The Treatment of Sarcoidosis with Chloroquine*

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MANY agents, including isoniazid, nitrogen mustard and calciferol, have been utilized in the treatment of sarcoidosis. None of these has been shown to be consistently effective. Adrenal cortical steroids remain the only therapeutic agents which are of demonstrable value. However, because of potentially serious side effects, their use in sarcoidosis is usually restricted to patients with life-threatening or organ-threatening disease.

Recently, we have noted that a few patients with documented sarcoidosis have positive latex fixation tests [*1*]. This finding suggested that sarcoidosis might be related in some way to rheumatoid arthritis or systemic lupus erythematosus. The antimalarial drugs Atabrine® and chloroquine are useful adjuncts in the therapy of these two disorders; furthermore, there have been two case reports indicating that Atabrine also promotes regression of the lesions of cutaneous sarcoidosis [*2,3*].

For these reasons we have undertaken a systematic study of the effect of chloroquine on the clinical course of patients with sarcoidosis. Results of one aspect of this program are presented in this report. These observations reveal that chloroquine, like Atabrine, is of distinct value in the treatment of cutaneous sarcoidosis. In addition, there is evidence that the administration of chloroquine results in the regression of extracutaneous manifestations of sarcoidosis.

Sarcoidosis is a disease which frequently undergoes spontaneous remission in its early stages. However, in the chronic stage (over two years' duration), spontaneous remissions, particularly of cutaneous lesions, are rare [*4,5*]. The studies reported herein were performed in seven patients who had documented sarcoidosis for more than two years. In order to ascertain accurately the minimum duration of illness, patients were selected who had cutaneous lesions as well as other evidence of sarcoidosis.

The diagnosis of sarcoidosis in these cases was made on the basis of the clinical course, tissue biopsy, and the response to a highly specific Kveim antigen [*6*]. As indicated in Table 1, biopsy specimen of the tissue was positive in the six patients in whom biopsy was performed; in the seventh patient the Kveim test had a positive reaction, as it did in five of the six who had positive biopsy specimens. None of the seven patients reacted to second strength Purified Protein Derivative. Significant hematologic abnormalities were not observed, nor was hypercalcemia present. In no patient in this group were the results of the latex fixation test positive.

After a suitable period of observation, chloroquine phosphate† was administered at a dosage of 500 mg. per day, usually for a period of six months. The subjects were seen at frequent intervals and their clinical status was evaluated, and appropriate roentgenograms and laboratory determinations were performed.

CASE REPORTS

CASE 1. In 1955 M. B., a thirty-six year old Negro woman, had cutaneous lesions of the arms and legs associated with hoarseness, a non-productive cough, and an abnormal roentgenogram of the chest. At that time histologic examination of a skin lesion showed changes typical of sarcoidosis. When seen at the Rockefeller Institute Hospital in 1957 she was found to have raised, hyperpigmented, irregular 0.7 to 1cm. lesions scattered over the neck, face and upper arms. Smaller nodular lesions were present on the eyelids, and when biopsy specimens were obtained were found to contain non-caseating epithelioid tubercles. There was generalized lymphadenopathy. A mixed hyper-

† Kindly supplied as Aralen® by Winthrop Laboratories, New York, New York.

* From The Rockefeller Institute, New York, New York.

trophic-atrophic nasopharyngitis extended to and involved the true vocal cords. Minimal evidence of interstitial fibrotic densities was noted on roentgenograms of the chest. The erythrocyte sedimentation rate (ESR, Westergren) was 9 mm. per hour, and the gamma globulin was 100 Zinc-2 units (upper limit of normal = 55) [7].

One month after the initiation of chloroquine therapy the skin lesions appeared flatter, the pharyngeal mucosa was less inflamed, and the patient's voice was more resonant. Progressive improvement continued and at the end of six months the only remnants of the skin lesions were small flat areas of hyperpigmentation. There was no evidence of mucosal abnormality. In addition, the gamma globulin had fallen to 70 Zinc-2 units.

The patient was then lost to follow up for ten months. When next seen the skin lesions had recurred. Chloroquine therapy was reinstituted for another six months, and the cutaneous lesions again slowly regressed. The patient has received no therapy for the past eight months and thus far there has been no relapse.

Case II. M. L., a forty-five year old Caucasian widow, had cutaneous lesions with the histologic characteristics of sarcoidosis fifteen years before her first clinic visit to the Rockefeller Institute Hospital. She had been treated in the past with local applications of hydrocortisone, galvanic stimulation, and dry ice without improvement.

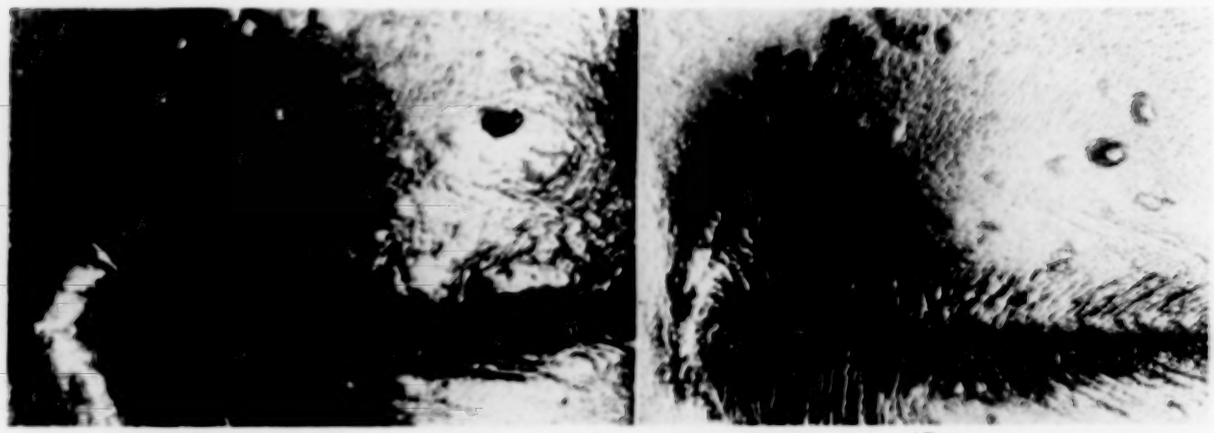
When first seen in February 1958, she had roughened, thick and vascular skin over the right cheek. There were heaped-up, granular, erythematous lesions over both nasal alae near the muco-cutaneous junction and at the tip of the nose. The remainder of the physical examination was within normal limits. The roentgenogram of the chest revealed disseminated, fibrotic nodulation throughout both lung fields with moderate enlargement of the hilar areas. The ESR was 55 mm. per hour and the gamma globulin was 61 Zinc-2 units.

During the next twelve months the patient was treated with isoniazid and local applications of hydrocortisone ointment. However, there was no clinical improvement. Chloroquine therapy was started in February 1959 and during the next several months there was progressive regression of the skin lesions. The granular and erythematous areas became flat and moderately smooth without discoloration. There was slight improvement in the roentgenographic appearance of the chest. During this first course of therapy the ESR fell to 30 mm. per hour and the gamma globulin level became normal (44 Zinc-2 units). One month after cessation of chloroquine therapy, the skin lesions became worse and within ten weeks they were at the same level as before treatment. Chloroquine therapy was reinstituted and again the lesions responded to treatment. Administration of the drug was discontinued after six months, and once again a relapse occurred within two months.

Case III. E. M., a thirty-two year old Negro woman, was diagnosed as having sarcoidosis seven years ago on the basis of hilar and superior mediastinal lymphadenopathy, a papular eruption on the face, and a positive reaction to a Kveim test. Her condition remained essentially unchanged except for the appearance of additional skin lesions on the anterior aspect of the lower legs five years ago. She was first seen at the Rockefeller Institute Hospital two years ago at which time firm, elevated pale papules were observed on the upper eyelid, on the bridge of the nose, and on the right patellar regions. Roentgenogram of the chest revealed marked bilateral hilar and superior mediastinal lymphadenopathy and patchy parenchymal infiltration in the right middle and both lower lung fields. The ESR was 34 mm. per hour, and the gamma globulin was elevated (77.5 Zinc-2 units).

She was treated for three months with the administration of 20 mg. of prednisone daily, and then the dosage was reduced to 15 mg. for the next five months. The skin and lung lesions did not change appreciably on steroid therapy and the sedimentation rate and gamma globulin levels remained elevated. Chloroquine was then given for a period of seven months. Within one month the skin lesions had diminished in size and at the end of the period of therapy they had disappeared. The intrathoracic lymphadenopathy and pulmonary infiltrations also improved. Whereas the ESR fell to a normal value of 10 mm. per hour, the gamma globulin level remained elevated (70 Zinc-2 units). Approximately two months after chloroquine therapy was discontinued, the facial skin lesions reappeared, and the pulmonary changes returned to the same state as before treatment. A new course of chloroquine will be instituted in the near future.

Case IV. R. R., a twenty-eight year old Negro woman, became ill four years ago when she was noted to have bilateral hilar lymphadenopathy and pulmonary infiltration with negative results of a tuberculin test. Three years ago she experienced transient bilateral parotitis and also noted the onset of skin lesions on both eyelids. She was seen at the Rockefeller Institute Hospital shortly thereafter and the following were found: multiple pale pink umbilicated papules on both upper eyelids, bilateral enlargement of the lachrymal glands, generalized lymphadenopathy, splenomegaly, enlargement of mediastinal and hilar nodes, elevated ESR (70 mm. per hour) and elevated gamma globulin (70 Zinc-2 units). A biopsy of the lymph node and reaction to the Kveim test confirmed the diagnosis of sarcoidosis. During the next eighteen months she was observed at regular intervals and her status remained essentially unchanged except for gradual diminution in splenomegaly and peripheral lymphadenopathy.



1A 1B

FIG. 1. Case IV. Cutaneous sarcoid lesions surrounding the left eye. A, immediately prior to treatment. B, after six months of chloroquine therapy.

2A 2B

FIG. 2. Case V. Perinasal lesions. A, prior to therapy. B, after three months of chloroquine therapy.

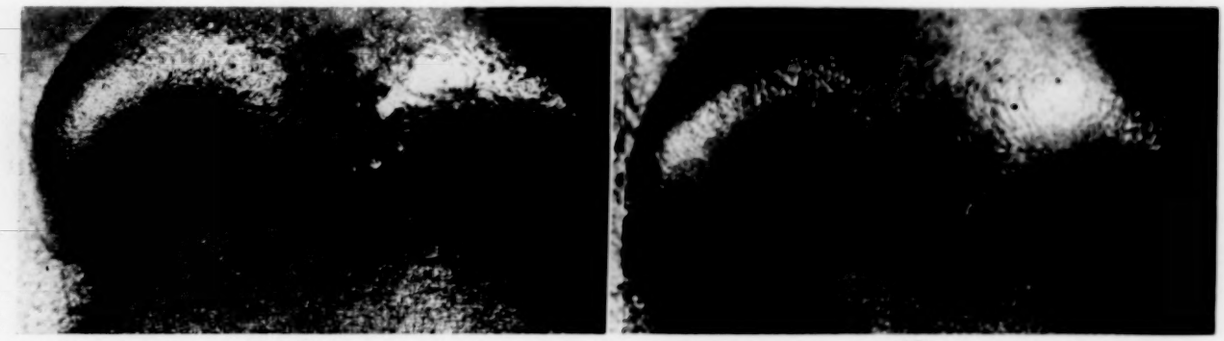
During a seven month course of chloroquine therapy there was a steady shrinkage of the skin lesions with hyperpigmentation at the site of resorption of the papules. (Fig. 1.) The peripheral lymphadenopathy and enlargement of the lachrymal gland also disappeared. The intrathoracic abnormalities did not change detectably during the course of therapy, but the ESR fell to normal and the gamma globulin level decreased to a nearly normal value of 59 Zinc-2 units. The time that has elapsed since discontinuation of chloroquine is not sufficient to determine whether or not a relapse will occur.

CASE V. M. T., a thirty-nine year old Negro woman, had persistent maculopapular facial lesions fifteen years ago. Nine years ago bilateral hilar lymphadenopathy was noted. Biopsy of the skin lesions showed the histologic features of sarcoidosis.

When seen at the Rockefeller Institute Hospital in 1958, the patient was noted to have numerous papular lesions on a vitiliginous area surrounding the nose. There were also papular lesions on the eyelids, cheeks, and the back of the neck. There was bilateral hilar lymphadenopathy and the ESR was 51 mm. per hour. The gamma globulin was 89 Zinc-2 units. During the next four months without therapy, the skin lesions fluctuated in size but remained prominent. Chloroquine was administered for seven months and the cutaneous lesions virtually disappeared. (Fig. 2.) The ESR fell to 35 mm. per hour and the gamma globulin to 59 Zinc-2 units. There was also some diminution in the size of the hilar nodes.

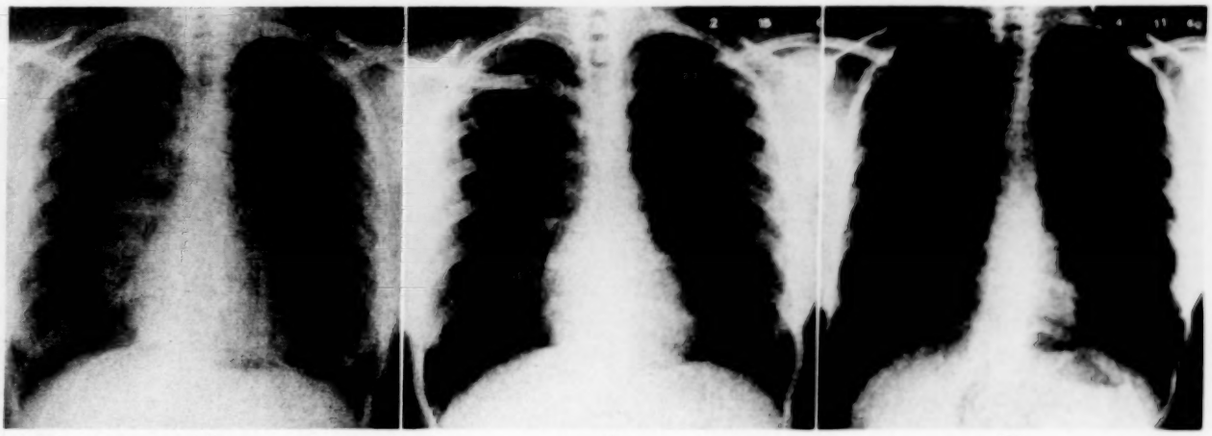
CASE VI. C. W., a forty-one year old Negro woman, had transient acute bilateral uveitis, generalized skin lesions, and a chest abnormality was observed on roentgenograms taken five years ago. She had been treated with adrenal cortical hormones for one year without effect.

When seen at the Rockefeller Institute Hospital in March 1960 she was found to have 1 to 5 cm. elevated lesions with heaped-up margins located on the trunk,



3A 3B

FIG. 3. Case VII. Nasal lesions. A, before chloroquine therapy. B, after five months of chloroquine therapy.



4A 4B 4C

FIG. 4. Case VII. Roentgenograms of the chest. A, immediately prior to the administration of chloroquine. B, after four months of chloroquine therapy. C, after five and a half months of therapy.

legs and upper arms. On the lower torso these lesions were virtually confluent. In addition, there were nodulations about the eyelids and mouth. Biopsy of the skin lesions showed changes compatible with sarcoidosis. There was evidence of bilateral uveal scarring, and there were fibrotic densities in both midlung fields. The ESR was 84 mm. per hour and the Zinc-2 was 77.

At the present time the patient has received chloroquine for only three months but there has been striking diminution in the size of the cutaneous lesions.

CASE VII. Eleven years ago D. W., a thirty year old Negro woman, was told that an abnormality was observed on her roentgenogram of the chest. Five years ago she was found to have generalized lymphadenopathy and hilar and mediastinal lymphadenopathy. Results of a Kveim test were positive. Two and a half years ago a non-productive cough, rhinitis, and partial nasal obstruction developed. In addition, papular lesions appeared at the tip of the nose and nodular lesions occurred on the left eyelid; histologic examination of a biopsy specimen of a lesion on the left eyelid revealed changes compatible with sarcoidosis.

When the patient was first seen at the Rockefeller Institute Hospital in July 1959 the following abnormalities were noted: a hypertrophic rhinitis occluding the left nasal passage, nodular and plaque lesions of the tip of the nose, and minimal cervical and submandibular lymphadenopathy. There was massive hilar and superior mediastinal lymphadenopathy which was essentially the same as that observed on a roentgenogram of the chest obtained in 1955. In addition, there was a reticulonodular infiltrate throughout both lung fields. The ESR was 47 mm. per hour and the gamma globulin was 67 Zinc-2 units.

One month after the initiation of chloroquine therapy the nasal obstruction had diminished and there was discernible improvement of the skin lesions. During the next five months, the skin lesions completely disappeared leaving flat areas of deeper pigmentation. (Fig. 3.) The nasal mucosa appeared normal. Concomitant with the improvement of the skin lesions, there was striking diminution in the size of the hilar and mediastinal lymphadenopathy; furthermore, the interstitial pulmonary densities disappeared. (Fig. 4.) The ESR fell to the normal value of 16 mm. per hour, and the gamma globulin to the normal value of 43 Zinc-2 units.

TABLE 1

THE CLINICAL STATUS AND RESPONSE OF PATIENTS WITH SARCOIDOSIS TO THE ADMINISTRATION OF CHLOROQUINE

Patient	Histopathology		Duration of Disease (yr.)	Abnormality and Response to Chloroquine						
	Biopsy	Kveim Test		Skin	Mucosa	Peripheral Nodes	Thoracic Nodes	Pulmonary Parenchyma	Erythrocyte Sedimentation Rate	Gamma Globulin
M. B.	Positive	Positive	2½	+	+	+	−	0	−	+
M. L.	Positive	Positive	16	+	−	−	±	±	+	+
E. M.	Not carried out	Positive	6	+	−	−	±	±	+	0
R. R.	Positive	Positive	4	+	−	+	0	−	+	+
M. T.	Positive	Negative	14	+	−	−	±	−	±	+
C. W.*	Positive	Positive	5	+	−	−	±	±	0	0
D. W.	Positive	Positive	10	+	+	−	+	+	+	+

NOTE: + indicates marked improvement or return to normal; ±, slight improvement; 0, no change; and −, normal or not involved.

* On therapy only three months.

One month after the cessation of chloroquine therapy, the hilar lymphadenopathy increased and within two months the cutaneous lesions had recurred. The ESR rose to 42 mm. per hour.

RESULTS

As indicated in the case reports and in Table 1, the administration of chloroquine was associated with marked improvement or clearing of the cutaneous sarcoid lesions in all patients. The response was frequently evident within one or two months, but maximal therapeutic effect often required six months of treatment. A residual area of hyperpigmentation was usually present at the site of resorbed papules and nodules. It is of interest that two of the patients in this group had previously received systemic therapy with adrenal steroids without benefit. Two other patients did not respond to topical therapy with adrenal cortical hormones.

Peripheral lymphadenopathy, which was present in two patients, disappeared during the course of treatment. In addition, thoracic nodes regressed to a variable extent in five of the six patients who had this abnormality. There was also some improvement in pulmonary lesions.

Two patients had involvement of the mucous membrane and in one of these (M. B.) virtually the entire nasopharyngeal mucosa was abnormal. With chloroquine therapy, both patients showed striking improvement. An additional patient not included in this series was of interest in this regard. She presented a localized sarcoid reaction of the lip of five years' duration. A Kveim test had negative results, and there was no evidence of systemic disease. Nevertheless, the administration of chloroquine brought about virtually complete regression of the lesions.

In five patients who had received chloroquine for at least six months and who initially had high sedimentation rates, the ESR returned to normal, as did elevations of the gamma globulin fraction of the serum.

Four patients (M. B., M. L., E. M., and D. W.) experienced clinical relapses within three months after cessation of chloroquine therapy. Two of the patients were given a second course of chloroquine and the lesions once again receded. A relapse has again occurred in one of these two subjects.

The mechanism by which chloroquine therapy causes the regression of sarcoid lesions is unclear. In view of the high relapse rate, it seems likely that the action of chloroquine is upon the inflammatory reaction, rather than upon the basic incitant of sarcoidosis. Since chloroquine is accumulated in leukocytes [*8*], the site of drug action may be upon the mononuclear, epithelioid and giant cells which comprise the inflammatory response in sarcoidosis.

The administration of chloroquine was associated with transient toxic manifestations (e.g.,

nausea, headache, blurred vision) in some patients. However, these symptoms disappeared within a few days, and discontinuation of the drug was unnecessary. One patient (M. T.) who has received chloroquine for seven months shows early signs of corneal opacity and the drug has therefore been withdrawn.

In view of the usually transient and reversible side effects of chloroquine, it is a relatively safe drug to administer provided that clinical follow up is frequent. It is certainly less hazardous than the administration of adrenal cortical hormones.

There are two major factors which indicate that the responses to chloroquine observed in this group of patients were not fortuitous. First, the chronic phase of sarcoidosis exhibited by the patients reported herein is not usually subject to spontaneous remission [*4,5*]. Secondly, the remissions and relapses clearly paralleled the administration and withdrawal of chloroquine. This is substantiated by the occurrence of second remissions during the administration of chloroquine to patients who had relapsed after a first course of therapy.

Evaluation of therapy in patients with acute sarcoidosis poses an entirely different problem which clearly calls for long-term, controlled observations. Such studies are now in progress but the final results will not be available for some time. Preliminary observations support the thesis that chloroquine is frequently an effective drug in the treatment of sarcoidosis.

SUMMARY

Chloroquine has been administered to seven patients with chronic sarcoidosis. In each instance there was considerable improvement of the cutaneous lesions. Regression of extracutaneous lesions was more variable, but improvement was often observed; this was particularly true of mucous membrane and thoracic node involvement. Associated with the clinical response, elevated sedimentation rates and high gamma globulin levels returned toward normal. It is suggested that chloroquine may be a useful drug for the treatment of many patients with sarcoidosis, and further studies are in progress to define the situations in which its use is of most benefit.

ADDENDUM

After this manuscript was submitted, reports have been published which also suggest that therapy with Atabrine or chloroquine may be of value in patients with sarcoidosis [*9,10*].

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An Analysis of Forty-Two Cases of Laboratory-Acquired Tularemia*

Treatment with Broad Spectrum Antibiotics

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THE hazard of infection with Pasteurella tularensis in laboratory workers is well recognized [*1*]; few persons escape illness if they continue to work with the organism. From August 1956 to February 1959 thirty-four patients with laboratory-acquired typhoidal tularemia were hospitalized and eight cases were detected in non-hospitalized personnel. These cases were caused by streptomycin-sensitive and streptomycin-resistant strains of P. tularensis, the latter being of laboratory origin [*2,3*].

The clinical and laboratory courses of these workers were documented according to a protocol initiated at the onset of this study with three primary objectives: (1) to evaluate the clinical and laboratory manifestations of the disease and attempt to establish criteria to achieve an earlier diagnosis; (2) to assess the efficacy of killed tularemia vaccines (phenolized [*4*] and acetone-extracted [*5*]) in the prevention or modification of the disease; and (3) to determine the effectiveness of tetracycline as a therapeutic agent.

METHODS

During hospitalization symptoms and signs were noted and graded daily as to severity. Prior to the initiation of antibiotic therapy each case was evaluated as to the severity of illness and graded as mild, moderate or severe. The illness was considered *mild* if the symptom-complex permitted the patient to work throughout the day but afternoon fatigue, chilliness, slight fever and malaise were noted; *moderate* if the patient was unable to work and bed rest for part of the day was desired; and *severe* if the patient was confined to bed. Rectal temperatures and vital signs were recorded every four hours.

Supportive therapy consisted of bed rest, as desired by the patient, and the administration of codeine for the more severe headache and myalgia; antipyretics were intentionally withheld. Isolation procedures were not practiced.

Routine blood cell count with differential, sedimentation rate (Wintrobe), C-reactive protein (CRP) and chest roentgenogram were obtained two to three times weekly during hospitalization. Attempts to isolate P. tularensis were made routinely from various clinical specimens: Fasting gastric aspirates during the first two or three days of hospitalization; pharyngeal washings (using 15 ml. nutrient broth as gargle) for the first three days; sputum specimens when a productive cough was present; and frequently blood. In certain cases bronchial washings, nasopharyngeal and throat swabs, and pleural fluid were examined.

Attempts to isolate P. tularensis from body fluids other than blood were made in the following manner: two 300 to 400 gm. male guinea pigs were inoculated with 1 ml. subcutaneously and 1 ml. intraperitoneally. Five-tenths to 2 ml. were plated on two to four glucose-cystine-blood (GCB) agar plates. Streptomycin (100 μg. per ml. of medium) was added to inhibit overgrowth of normal flora, thus permitting growth of streptomycin-resistant organisms. During the course of the study a modified GCB medium containing crystal violet (1 p.p.m.) and penicillin (100 to 500 units per ml.) was developed for similar reasons for isolation of streptomycin-sensitive organisms. Blood specimens were examined by giving a 4 ml.

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injection of a heparinized specimen to guinea pigs as already described, and by culturing 5 to 10 ml. in a diphasic medium containing GCB agar and broth, with observation continuing for thirty days.

Inoculated guinea pigs were observed daily for temperature elevation, roughing of the fur and hunching of the back. Deaths in "positive" animals occurred at three to seven days. At postmortem the findings were excessive, viscous, clear peritoneal fluid, a thickened greater omentum and a mucopurulent exudate around the spleen and liver. In animals surviving one week, the liver and spleen were greatly enlarged and a fine granular infiltrate was readily seen. A tentative diagnosis was made by staining smears of this exudate by Wayson and Gram methods. The exudate was cultured on GCB agar with and without streptomycin (100 μg. per ml.). Growth occurred on both plates if the organism was streptomycin-resistant, but only on plates without streptomycin if sensitive. Grey, translucent colonies, 1 to 2 mm. in diameter, were apparent at forty-eight to seventy-two hours and smears of these revealed gram-negative coccobacilli. Identification was made by slide agglutination using specific antiserum with appropriate controls.

At least one isolate from each case was examined for sensitivity to Chloromycetin® and the tetracyclines, using commercially prepared antibiotic discs. Sensitivity to tetracycline was further evaluated by determining growth on GCB agar plates containing 1 to 5 μg. of antibiotic per ml. When the organism did not grow in the presence of 100 μg. of streptomycin the sensitivity was determined against 10 μg. per ml.

Specific agglutinin titers, using doubling dilutions of serum, were measured at least weekly during the early phase of disease and on each follow-up visit. As a part of another study by one of us [6], serial hemagglutinin levels were obtained from fifteen patients. A hemagglutination-inhibition method was used in attempts to detect the P. tularensis polysaccharide in various body fluids collected from fourteen patients.

Tularemia skin tests were performed shortly after admission and usually weekly thereafter until a positive reaction occurred, using 0.1 ml. of phenolized vaccine diluted 1:1000 with physiologic saline solution injected intradermally into the flexor surface of the forearm. The reaction to the skin test was considered positive if erythema and edema of 10 mm. or more were present in any one diameter at forty-eight hours.

After hospitalization each patient returned to the out-patient department for weekly follow-up evaluations during the first month, monthly for the next three months and every third month to complete a year. Each evaluation included interval history, physical examination when indicated, complete blood count, sedimentation rate, CRP, agglutinin titer and chest roentgenogram.

MATERIAL

The forty-two patients were between twenty-one and forty-five years of age, except one, who was sixty-five. There were forty-one men and one woman; nine (eight men and one woman) were Negroes. All were in good health prior to the onset of disease and none had a history of naturally occurring tularemia.

All but one patient had received some quantity of phenolized or acetone-extracted P. tularensis vaccine. As an initial course three subcutaneous injections of vaccine were given, 0.25 ml. the first day, and 0.5 ml. on each of the next two days. Booster injections consisted of 0.25 and 0.5 ml. on two consecutive days, given six months to a year following the initial series, with additional boosters as deemed necessary if the reaction to the skin test was negative. The initial vaccination had been performed from less than one month to as long as eleven years prior to the onset of illness. (Tables I and II.) Thirty-one patients had been given one to seven booster injections, with approximately 90 per cent receiving four or less. Ten had been given an initial series only, ranging from one month to as long as five and a half years prior to infection. Fifteen of the former group of thirty-one and seven of the latter ten became ill less than six months after vaccination. All but three patients were known to have a tularemia agglutinin titer at some time before infection; in 80 per cent it had been determined within six months of illness.

Occupational activities resulted in frequent potential respiratory exposure to P. tularensis in most of these subjects. In twenty, specific accidents occurred, permitting an estimation of incubation periods. In sixteen, the presumed incubation period was three to six days; in the remaining four, seven to twelve days.

Clinical Observations in Hospitalized Patients. On the basis of the subjective criteria previously described, the illness was considered severe in eight people (four of them Negroes), moderate in ten and mild in sixteen. (Table I.) This division was further emphasized when the day of hospital admission was compared to the day of illness: the severely ill patients were admitted within the first week of illness, whereas over half of the mild to moderately ill patients were seen initially after the first week. Presumably such patients did not seek medical attention until persistence of the mild symptoms became disturbing. Of equal importance was the inability of the physician to differentiate the symptoms of tularemia from common grippal states. An influenza epidemic during which half of the group became ill with tularemia complicated recognition. In the absence of acute symptoms, suspects were followed closely in the out-patient clinic. Individuals were hospitalized if there were persistence of symptoms or if x-ray evidence of pulmonary involvement was found.

A grippal symptom-complex of varying severity was seen and the high incidence of respiratory symptoms

TABLE 1

SUMMARY OF THIRTY-FOUR HOSPITALIZED PATIENTS WITH TYPHOIDAL TULAREMIA WITH RESPECT TO SEVERITY, VACCINE ADMINISTRATION AND AGGLUTININ TITER (1956–1959)

Case No.	Year	Severity*	Vaccine Administration			Specific Agglutinins						
			Initial Series (months before onset)	Boosters		Pre-infection Level		First Obtained During Illness		Fourfold Rise (day of illness)	Maximum	
				No.	Months Before Onset	Titer†	Days Before Onset	Titer	Day		Titer	Day of Illness
Patients with Pulmonary Involvement												
1	1956	Mild	66	1	54	40	75	80	8	26	2,560	40
2	1956	Mild	8	1	3	20	3	40	3	23	1,280	23
3	1956	Moderate	65	0	...	80	101	80	4	30	2,560	46
4	1956	Mild	5	0	...	80	24	320	3	6	2,560	9
5	1957	Mild	91	5	33	160	61	160	10	None	1,280	31
6	1957	Mild	37	1	26	80	60	40	2	29	640	29
7	1957	Mild	40	3	<1	80	114	80	6	34	1,280	34
8	1957	Moderate	103	6	6	20	187	40	5	23	2,560	43
9	1957	Moderate	56	1	3½	0	127	160	7	18	2,560	18
10	1957	Mild	50	2	1	40	58	40	6	34	1,280	49
11	1956	Severe	14	1	6	320	28	1,280	6	None	1,280	6
	1957	Moderate	36	1	29	160	197	160	1	None	1,280	60
12	1957	Mild	36	3	2	20	54	40	3	19	1,280	28
13	1958	Moderate	43	1	11	160	153	40	3	30	640	30
14	1958	Severe	61	2	3	40	131	80	2	25	2,560	31
15	1958	Severe	81	3	49	10	106	0	1	11	2,560	28
16	1958	Mild	127	7	9	320	72	160	7	None	1,280	23
17	1958	Moderate	13	1	7	40	62	40	3	23	1,280	37
18	1958	Severe	63	3	4	20	122	40	6	23	2,560	40
19	1958	Moderate	54	3	1	40	30	80	14	30	1,280	30
20	1959	Severe	None	0	...	...	...	20	2	17	1,280	19
Patients Without Pulmonary Involvement												
21	1956	Moderate	26	1	18	80	4	80	9	33	1,280	33
22	1956	Mild	44	0		40	103	80	8	15	1,280	19
23	1956	Mild	16	1	14	160	9	80	5	19	2,560	34
24	1956	Mild	28	1	21	40	187	40	1	27	1,280	30
25	1957	Mild	48	0	...	40	96	20	1	20	2,560	27
26	1957	Severe	5	0	...	20	18	40	3	30	1,280	33
27	1957	Moderate	62½	1	51	0	53	10	5	16	640	31
28	1957	Severe	53	2	½	20	19	320	2	None	640	29
29	1957	Mild	59	0	...	40	43	40	10	21	2,560	40
30	1957	Severe	5	0	...	0	165	20	2	31	1,280	38
31	1958	Moderate	41	1	39½	20	129	10	5	12	1,280	22
32	1958	Severe	95	5	1½	160	7	80	2	None	640	60
33	1958	Mild	48½	1	40	10	13	10	3	19	1,280	36
34	1958	Mild	14	1	5½	20	199	10	3	25	320	25

* See text for definition.

† Expressed as the reciprocal of the highest serum dilution showing agglutination.

TABLE II

SUMMARIES OF EIGHT NON-HOSPITALIZED PATIENTS WITH TYPHOIDAL TULAREMIA WITH RESPECT TO VACCINE ADMINISTRATION, SYMPTOMS AND AGGLUTININ TITER (1956–1958)

Case No.	Year	Vaccine Administration			Symptoms		Tularemia Agglutinin Titer			
		Initial Series (months before onset)	Boosters		Type	Duration (days)	Base Line		Fourfold Rise	
			No.	Months Before Onset			Titer	Days Before Onset	Titer	Day of Illness
35	1957	1	0	...	Chills, night sweats, malaise, anorexia, fatigue	5–6	20	36	640 2,560	21 77
36	1958	80	4	4	Headache, lethargy, chilliness, anorexia	9–10	80	7*	1,280	38
37	1958	54	3	5	Slight fever, chilliness, dry cough, malaise	6	20	3*	2,560	35
38	1958	3	0	...	Coryza, cough, headache, sore throat	10	40	12	1,280	12
39	1957	5	0	...	Mild coryza	3	80	4	1,280	18
40	1957	84	3	10	Frontal headache, cough, low-grade fever, malaise	14†	20	154	1,280	41
41	1957	5	0	...	Headache, anorexia, nasal stuffiness	2–3	80	67	2,560	25
42	1957	17	1	...	Chills, slight fever, sore throat, slight cough, headache, back ache	6	40	140	1,280 2,560	3 7

* Pre-illness serum not available. Number given represents the day *after* onset of illness in this instance.
† 0.5 gm. streptomycin, given on the first, thirteenth and fourteenth days of disease.

was striking. All but four had one or more of the following, accompanying or appearing shortly after the onset (Fig. 1): a dry to slightly productive cough, minimal nasal stuffiness, a raw feeling of the throat and vague substernal tightness. With the exception of more frequent pleuritic chest pain and cough in the patients with radiographic evidence of pulmonary involvement, there was little difference in the incidence, severity or type of respiratory symptoms between the typhoidal and typhoidal-pulmonic groups. The presence or absence of x-ray evidence of pulmonary involvement could not be related to the severity of disease, with the exception of the one critically ill patient who had extensive pulmonary involvement.

The maximum rectal temperature usually occurred in the evening hours, ranging from 101° to 105.8°F., and generally paralleled the severity of illness. In half of the patients the fever did not exceed 103.0°F. Non-purulent pharyngitis and rhinitis were common. Chest abnormalities were limited to the group with positive findings on roentgenograms and consisted of signs of pleural fluid in five patients and of fine to coarse rales in five patients with bronchopneumonia. With the exception of transient splenomegaly in one patient and persistent submandibular adenopathy in another, there were no other pertinent physical findings.

Laboratory Observations in Hospitalized Patients. Among the twenty patients with positive findings on chest roentgenograms, pneumonic infiltrates were noted in seventeen, hilar adenopathy in nine, pleural effusion in five and perihilar linear streaking in one. The pneumonic lesion had a distinctive appearance; in twelve there was a single oval 2 to 8 cm. density with an indistinct border (Fig. 2A), in three there

FIG. 1. Principal symptoms and signs in thirty-four hospitalized patients with tularemia.

were similar but multiple lesions. There was no predisposition to any one lobe but in five the infiltrate was juxtahilar, merging with the hilar shadows. One patient had lobar consolidation (Fig. 2B), one had diffuse bronchopneumonia of one lobe. The availability of multiple chest films before the illness permitted critical evaluation of hilar shadows. The moderate, unilateral hilar enlargement was always associated with other abnormalities, occurring with a pneumonic infiltrate in eight and perihilar streaking with pleural effusion in the remaining patient. Pleural effusion was noted as an isolated occurrence in two patients.

The initial white blood cell count exceeded 14,000 per cu. mm. in only two patients. The differential count usually demonstrated a mild increase in young neutrophils and a few atypical lymphocytes. The sedimentation rate and the CRP were abnormal shortly after the onset of illness and paralleled one another during the acute phase.

A twofold or greater rise in the specific agglutinin titer was usually apparent by the third week of illness. A fourfold (considered diagnostic) rise was noted in four patients within the first two weeks and in twenty-four between the third to the fifth weeks from the onset of illness. In the remaining six patients only one- to threefold titer rises developed; diagnosis was confirmed in five by isolation of an organism and in one by typical lesions evident on chest roentgenograms and skin test conversion to positive. Before infection, these six patients had titers of 1:160 to 1:320, later showing a diagnostic rise (Table I), in contrast to the

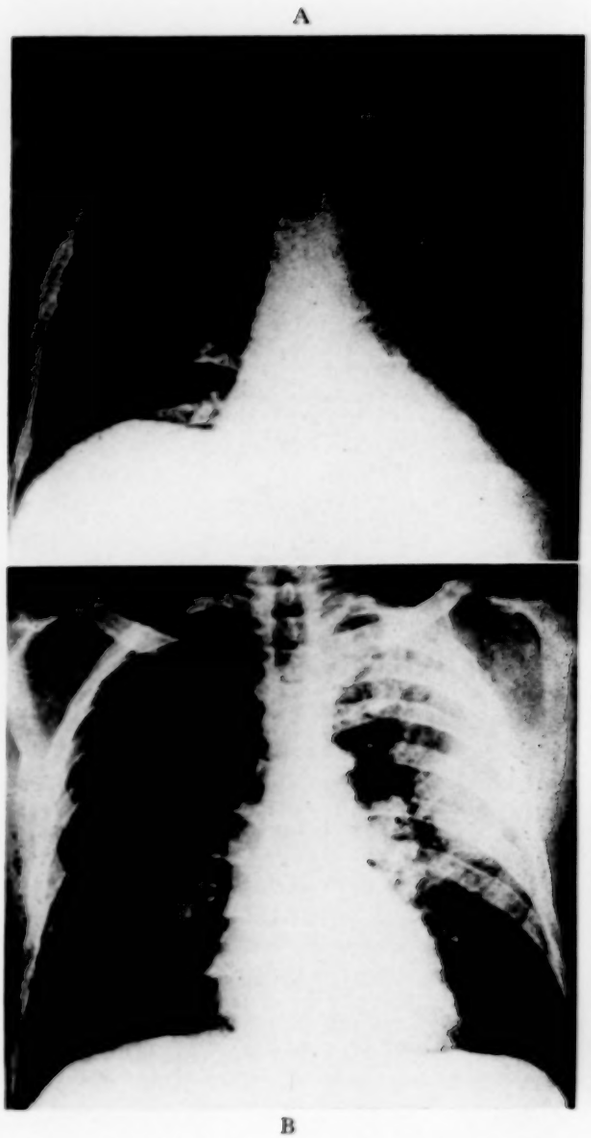

FIG. 2. A, Case 18, eighth day of illness. An oval infiltrate is evident in the upper lobe of the right lung. B, Case 15, eighth day of illness. Diffuse infiltrate is evident in the upper lobe of the left lung, with beginning consolidation and left hilar adenopathy.

twenty-seven patients who had titers of 0 to 1:80 before infection. The titer before infection in the unvaccinated subject was not known. Only 12 per cent of the patients showed low grade brucella agglutinin titer rises.

The first rise of hemagglutinin and agglutinin titers was detectable simultaneously in fifteen cases. The serum of ten patients showed a fourfold rise in hemagglutinins seven to ten days earlier than in agglutinins. In the remaining five the "diagnostic" rises were simultaneous. Attempts to identify the cellular polysaccharide of P. tularensis from other

TABLE III

ISOLATIONS FROM GASTRIC, PHARYNGEAL AND SPUTUM SPECIMENS IN THIRTY-THREE PATIENTS WITH TYPHOIDAL TULAREMIA

Specimen	Cases by Initial Week Tried						Total	
	1		2		3			
	No. Positive / No. Tried	%	No. Positive / No. Tried	%	No. Positive / No. Tried	%	No. Positive / No. Tried	%
Gastric	14/18	78	7/11	63	1/2	50	22/31	70
Pharyngeal	13/20	65	5/10	50	0/2	0	18/32	56
Sputum	8/9	89	5/6	83	1/1	100	14/16	88

body fluids by hemagglutination technics were not successful.

Skin tests were performed during illness in thirty-one patients; fourteen had a negative reaction to skin tests when first seen (eleven during the first week, three during the second week), which later converted to positive. The remaining seventeen had a positive reaction to skin tests shortly after admission (only four had a known positive reaction to skin tests prior to illness). Thus in approximately 58 per cent of the cases the skin test was not helpful in early diagnosis.

Table III shows the results of isolation attempts by guinea pig inoculation and/or culture on GCB agar from gastric, pharyngeal and sputum specimens from thirty-three patients with typhoidal tularemia. (No attempt was made in one patient.) Initial attempts to isolate the organism were made during the first, second and third weeks of disease in twenty, eleven and two patients, respectively. Fasting morning gastric aspirates were obtained from thirty-one patients and twenty-two yielded P. tularensis. The first gastric specimens from eighteen patients were positive; the second attempt yielded the first isolation from this material in only four additional cases. Subsequent gastric specimens were negative if the first two attempts were unsuccessful. Pharyngeal washings were positive in eighteen of thirty-two patients, the initial specimens yielding the best results, i.e., positive in fifteen. Sputum specimens from fourteen of the sixteen patients so examined were positive for P. tularensis; three of these did not have x-ray evidence of pulmonary involvement.

Sputum specimens were the only positive specimens for P. tularensis from two patients, a pharyngeal washing from one patient and gastric aspirates from six patients. Nineteen cases were confirmed by positivity from at least two of these three sources.

Other specimens examined included bronchial lavage (positive in two of three patients) and nasal swabs (positive in one of eight); the positive specimens in these instances were from patients whose gastric or pharyngeal washings also yielded P. tularensis. The organisms were recovered from the blood of only two, one critically ill and one during a second relapse. In summary, the organism was isolated from gastric, pharyngeal or sputum samples in twenty-eight of thirty-three cases of typhoidal tularemia.

All isolates tested were sensitive to 5 μg. of the tetracyclines and of Chloromycetin. In addition, inhibition of growth of each isolate was demonstrated on GCB agar containing as little as 1 μg. of tetracycline per milliliter of medium. The isolates from twenty-one of the twenty-eight, grew readily in the presence of 100 μg. of streptomycin, six did not. From one patient both sensitive and resistant strains of P. tularensis were isolated.

Thirty-two of the thirty-four hospitalized patients were treated (Table IV) with broad spectrum antibiotics given every six hours in equally divided doses. Tetracycline was given orally to twenty-eight patients: 2 gm. daily to twenty-six, 1 gm. daily to two. Sixteen of these twenty-eight patients received an initial 1 to 2 gm. "loading dose." Three patients were given other broad spectrum antibiotics: one (Case 24) 2 gm. daily of chlortetracycline; one (Case 22) 2 gm. daily for ten days of Chloromycetin followed eighteen days later by 2 gm. daily (ten days) of novobiocin; and one (Case 15), 1.5 gm. daily for five days of intravenously administered oxytetracycline, followed by 3 gm. daily of orally administered tetracycline to complete twenty-one days. One patient (Case 25) received tetracycline and streptomycin (2 gm. daily) for ten days.

Therapy was instituted during the first, second and third week of illness in fifteen, twelve and five patients, respectively. A summary of duration of antibiotic therapy is shown in Table V. In all cases there was a striking fall in fever within the first twenty-four hours, only four patients having rectal temperatures exceeding 101.0°F. after forty-eight hours. The mean dura-

TABLE IV

RESPONSE TO ANTIBIOTIC TREATMENT IN THIRTY-FOUR HOSPITALIZED PATIENTS WITH TULAREMIA INCLUDING SENSITIVITY TO STREPTOMYCIN

Severity*	Case No.	Broad Spectrum Therapy: Initiation (day of disease)	Broad Spectrum Therapy: Loading Dose	Broad Spectrum Therapy: Duration (days)	Temperature >100°F. (R) (post-therapy in days)	Streptomycin Sensitivity of Isolate and Other Comments
Typhoidal with Pulmonary Involvement						
Mild	4	None	−	None	4†	Resistant
	2	10	−	10	0	No isolation
	1	15	−	10	1	Resistant
	7	15	−	13	1	Sensitive
	6	4	+	16	2	Resistant
	5	9	+	14	2	Resistant
	16	10	+	11	3	No isolation
	12	17	+	11	3	Sensitive
	10	10	+	21	16	Sensitive
Moderate	3	7	−	7	1	Resistant
		None	−	None	17†	Untreated relapse
	19	16	−	14	1	Resistant
	13	6	+	10	2	Resistant
	11	9	+	10	2	Sensitive (reinfection)
		6‡	−	11	2	Resistant (1st infection)
	17	5	+	11	3	Sensitive
		30	−	13	3	Treated relapse
	9	9	+	14	4	Resistant
	8	9	+	8	4	Resistant
Severe	14	3	+	7	2	Sensitive
		25	−	13	2	Treated relapse
	18	6	−	14	3	Resistant
	20	2	−	14	3	Sensitive and resistant
		25	+	10	2	Treated relapse
		79	−	14	0	Treated relapse
	15	6	+	21	16	Resistant
Typhoidal Without Pulmonary Involvement						
Mild	33	None	−	None	3†	No isolation
	23	9	−	7	1	Resistant
	34	2	+	14	2	Resistant
	29	14	+	10	4	Resistant
	25	20‡	−	27	4	No isolation
	24	14	−	7	6	No isolation
	22	12	−	10	7	Resistant
Moderate	31	5	+	11	2	Resistant
	27	6	+	10	2	Resistant
	21	10	−	10	2	No attempt to isolate
Severe	26	6	−	10	1	Resistant
		31	−	15	5	Treated relapse
	28	2	−	6	2	Resistant
	32	3	+	11	2	Resistant
	30	6	+	11	2	Resistant

* See text for definition.
† Total days of fever.
‡ Broad spectrum therapy with added streptomycin.

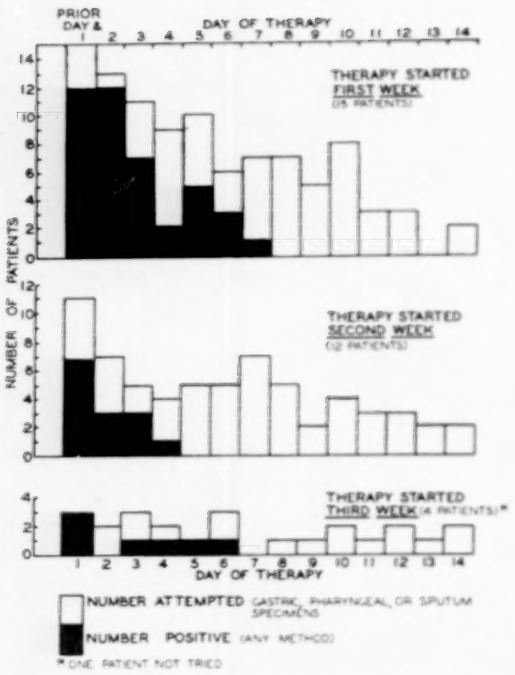

FIG. 3. Status of isolation attempts in tularemia patients treated with broad spectrum antibiotics only.

tion of any rectal temperature elevation above 100.0°F. (Table IV) following onset of therapy was approximately three days (the first day of therapy is included in this mean). There was a corresponding improvement in symptoms within the first forty-eight hours. The response was equally rapid in the eight patients categorized as being severely ill and in the ten patients who did not receive a "loading dose" of tetracycline. Only two patients had a low grade fever exceeding one week after initiation of drug therapy. One of these (Case 10) was mildly ill but had a persistent pleural effusion; the other was the critically ill patient with lobar pneumonia (Case 15). With these two exceptions, all patients were asymptomatic in less than one week after initiation of therapy. The erythrocyte sedimentation rate fell in the majority of patients and there was a return to normal of the CRP within two weeks following completion of drug therapy. Likewise, the lung lesions began to improve during the first week of treatment, thereafter slowly resolving and clearing by the fourth to sixth week in all but three patients. In one critically ill patient a linear infiltrate in the area of the previous lobar consolidation resolved during the following year. One patient had residual pleural thickening which gradually cleared over an eight-month period. In the third patient slight linear infiltrates persisted for three months in the two areas of bronchopneumonia which appeared during the second relapse.

The recovery rate of P. tularensis following the initiation of bacteriostatic drug therapy is demonstrated in Figure 3. The organism was commonly

TABLE V
DURATION OF THERAPY IN THIRTY-TWO PATIENTS

Therapy	No. of Patients — Duration of Therapy Days					
	6–8	10–11	13–14	16	21	Total
Tetracycline	5	14	7	1	1	28
Chlortetracycline	1	0	0	0	0	1
Chloromycetin	0	1	0	0	0	1
Oxytetracycline (I.V.) and tetracycline	0	0	0	0	1	1
Tetracycline and streptomycin	0	1	0	0	0	1
Total	6	16	7	1	2	32

isolated from sputum specimen, pharyngeal washings, gastric washings or combinations thereof throughout the first, but not the second, week of treatment, regardless of whether therapy was started during the first, second or third week of illness.

Eight patients had complicated clinical courses. In one (Case 22) a palpable submandibular lymph node developed on the twelfth day of illness which was eventually biopsied. Another (Case 10) had a slowly clearing pleural effusion with residual pleural thickening and dull pain in the right upper quadrant. The third (Case 15), who was critically ill, responded promptly to therapy but required a two-month convalescence. Five patients had excellent initial responses to the administration of tetracycline, followed by a recrudescence of disease one to two weeks after cessation of therapy. (Fig. 4.) In each instance the symptoms were less severe than during the initial illness.

One patient (Case 26) with typhoidal tularemia relapsed seven days after a ten-day course of tetracycline started on the sixth day of disease. Therapy was reinstituted on the ninth day of relapse.

One patient (Case 14) with typhoidal-pulmonic disease relapsed fourteen days after completion of seven days of tetracycline therapy begun on the third day of illness. Therapy was reinitiated on the second day of relapse.

One patient (Case 17) with typhoidal-pulmonic disease relapsed fourteen days after completing eleven days of tetracycline therapy instituted on the fifth day of disease. Therapy was reinstituted on the second day of relapse.

Another patient (Case 20) with typhoidal-pulmonic disease relapsed ten days after completing fourteen days of tetracycline therapy started on the second day of disease. Therapy was reinstituted on the second day of relapse. Six weeks after this course of therapy a second relapse occurred for which tetracycline therapy was started on the seventeenth day of a low grade fever.

Isolation attempts were successful during the re-

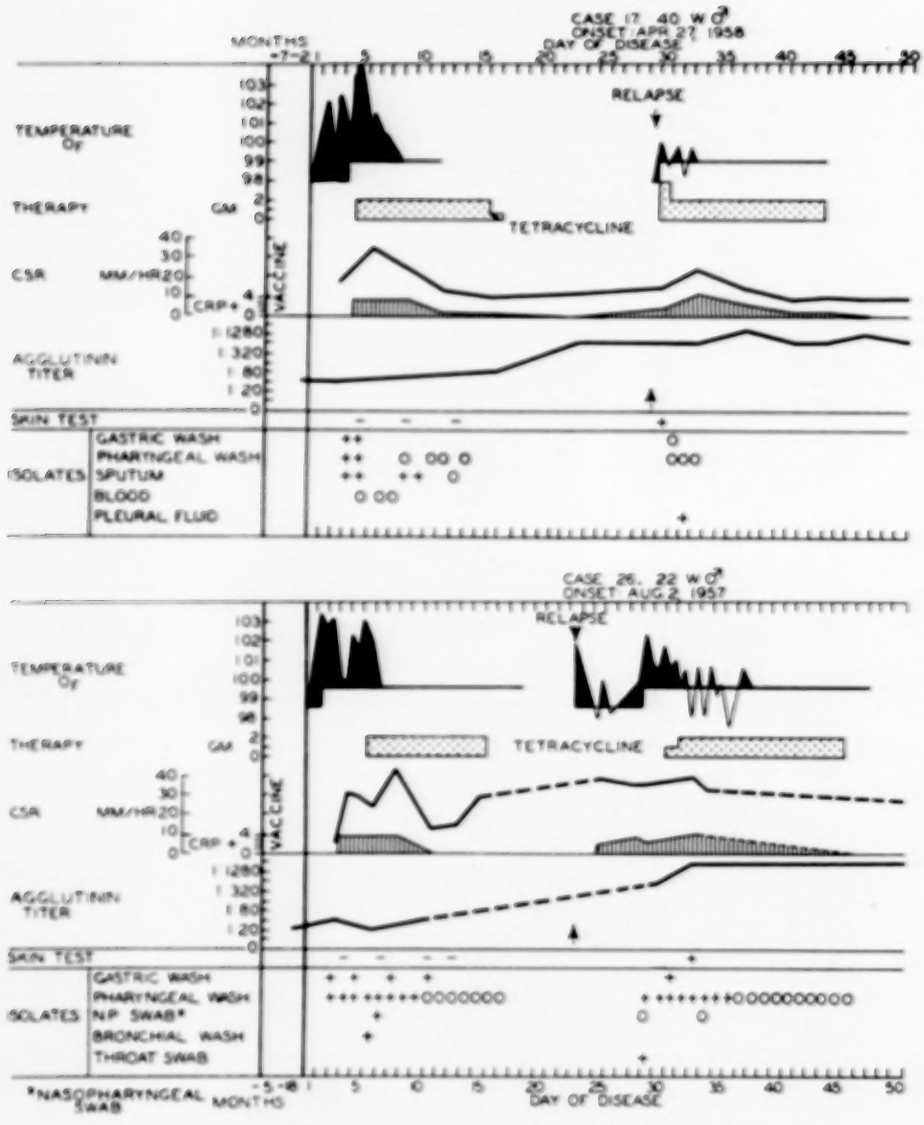

FIG. 4. Summaries of two relapse cases of tularemia.

lapses of three patients. In each relapse the isolates remained sensitive *in vitro* to tetracycline; a 2 or 3 gm. daily oral dose of tetracycline, given for ten to fourteen days, promptly controlled the disease.

One patient (Case 3) with typhoidal-pulmonic disease relapsed seven days after completion of a week of tetracycline therapy started on the seventh day of illness. No attempt was made to isolate the organism during this presumed relapse, nor was additional therapy given. The interrelationship of time of initiation and duration of therapy and relapse incidence is demonstrated in Table VI.

Fifteen of the thirty-one treated patients were treated with broad spectrum antibiotics within the first seven days of their illness; the five relapses occurred in this group. There were no recurrences in the sixteen patients who were treated similarly after the first week of illness. (Table VI.) Despite the absence of overt relapse in this latter group, four asymptomatic patients had rises in their sedimentation rates occurring at about the same time as the previously described clinical relapses. (Fig. 5.) This "subclinical" rebound also occurred in two patients in whom therapy had been initiated during the first week of illness.

Two hospitalized patients did not receive specific therapy. One (Case 4) was hospitalized on the fourth day of an illness characterized by low grade fever and mild grippal symptoms. He was asymptomatic the following day. The other (Case 33) complained of frontal headache, low back pain, weakness, moderate cough and occasional shaking chills of one week's duration. On the first hospital day his temperature

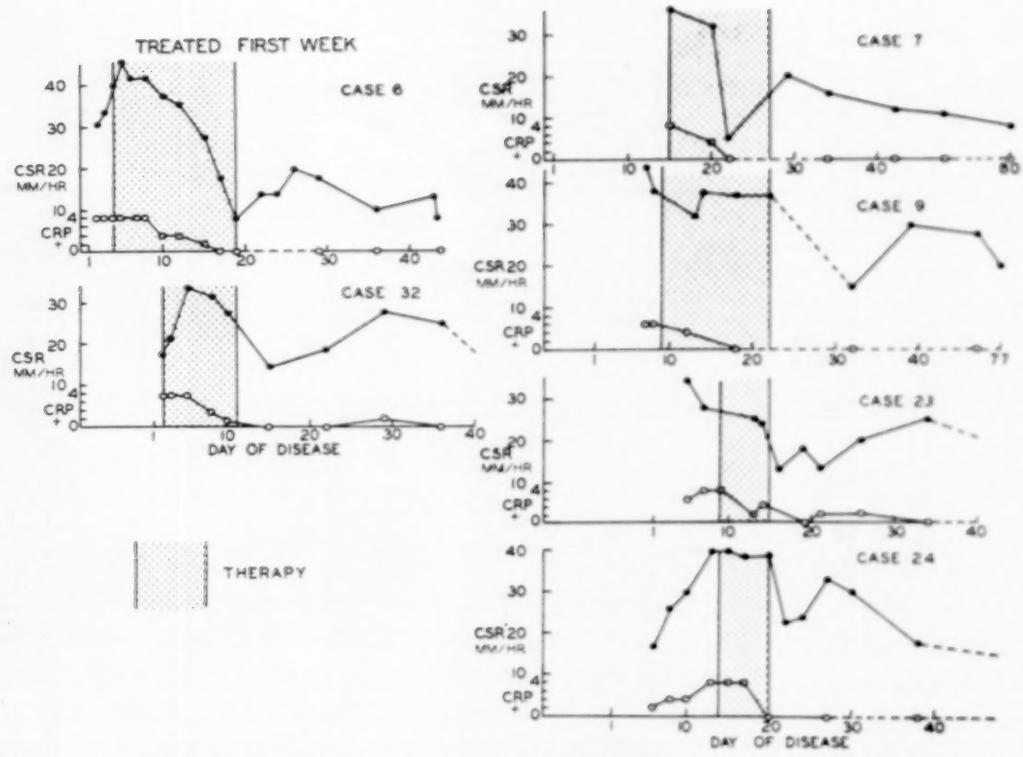

FIG. 5. Subclinical rebounds in six patients treated with tetracycline, 2 gm. per day (except Case 7, 1 gm. per day), as demonstrated by rises in sedimentation rate (corrected).

reached 104°F.; on the following day he was afebrile and asymptomatic. Chest films were within normal limits and attempts to isolate the organism were unsuccessful. A positive reaction to the skin test and a subsequent rise in tularemia agglutinating antibody from 1:10 to 1:1280 confirmed the diagnosis.

TABLE VI

RELAPSES AND SUBCLINICAL REBOUND* IN THIRTY-ONE PATIENTS WITH TULAREMIA WITH RESPECT TO TIME OF INITIATION AND DURATION OF BROAD SPECTRUM THERAPY

Duration of Therapy (days)	No. of Cases					
	Therapy Started First Week	Clinical Relapses	Subclinical Rebounds	Therapy Started Second or Third Week	Clinical Relapses	Subclinical Rebounds
6–8	3	2	0	3	0	2
10–11	7	2	1	7	0	0
13–14	3	1†	0	6	0	2
>14	2	0	1	0	0	0
Total...	15	5	2	16	0	4

* See text for definition.

† This patient relapsed again six weeks following the second course of therapy.

Non-hospitalized Patients. Routine follow-up tularemia agglutinin titers and skin tests are obtained after non-specific respiratory or grippal illness occurring in laboratory workers. Three cases (Cases 35, 36 and 37) were identified in this manner. (Table II.) The remaining five patients were not seen during their illness. One (Case 38) reported a potential exposure; blood was drawn and a tularemia antibody titer was reported as 1:40. At a follow-up evaluation twenty-three days later the titer was 1:1280 and the reaction to the skin test had converted to positive. Interval history indicated a mild respiratory illness of approximately ten days' duration which occurred twelve days following exposure. A fifth patient (Case 39) was identified by his participation in a vaccine evaluation study in which monthly agglutinin titers were obtained. Five months following the initial vaccine series his titer was known to be 1:80. Three weeks later it was 1:1280 and the reaction to the skin test was positive. An interval history revealed a mild respiratory illness of three days' duration occurring eighteen days before. Another patient (Case 40) was seen by his local physician and treated for possible sinusitis and bronchitis. He received 400,000 units of penicillin and 0.5 gm. of streptomycin on the first, thirteenth and fourteenth days of illness. Five months before this illness his titer was 1:20. There had been no intervening booster series of serial tularemia agglutinin titers. Six weeks after onset of his mild

bronchitis the tularemia titer was 1:1280 and the reaction to the skin test was positive. He was the only patient of the non-hospitalized group to receive any form of therapy. In the remaining two patients (Cases 41 and 42) a high titer and positive reaction to the skin test were noted at the time of evaluation for booster vaccination. Anamnesis revealed a mild respiratory illness from three to seven days' duration which had occurred during the preceding month and could have represented a mild episode of typhoidal tularemia. Thus the eight non-hospitalized patients had illnesses characterized by mild grippal symptoms.

Follow-up Examination. Approximately 80 per cent of the forty-two patients have been followed up for at least a year after their illnesses. Only three had residual complaints. One subject (Case 10) in whom therapy was started on the tenth day of illness continued to have a low grade fever for the first sixteen days of a twenty-one-day course of tetracycline therapy. The multiple, ovate, bronchopneumonic lesions promptly subsided, but the pleural reaction failed to clear completely until eight months later. This patient's only complaint was dull intermittent pain in the lower right posterior portion of the chest. A staghorn calculus of the right kidney was discovered seven months later; the lower chest pain subsided following nephrectomy. The remaining two patients with typhoidal tularemia (Cases 26 and 3) had clinical relapses following excellent responses to initial therapy. One patient (Case 26) promptly responded to a second course of tetracycline therapy. Nevertheless, he continued throughout the following year to complain of fatigability and generalized myalgia on damp days although physical examination, temperature, sedimentation rate and CRP remained within normal limits. The other patient was not treated during relapse and his low grade illness subsided in six months.

CASE REPORTS

Case 4 (Fig. 6). This twenty-seven year old Negro man was admitted November 14, 1956. Three days earlier he had noted intermittent chilliness, slight feverishness, and a daily oral temperature ranging from 100° to 101°F.

Five months prior to illness the patient had received an initial vaccine series for tularemia; the reaction to the skin test was negative at that time. The agglutinin titer twenty-five days before illness was 1:80.

Physical findings were limited to a few crepitant rales over the mid-portion of the left posterior lung field and a rectal temperature of 101.0°F. On the fifth day of illness he spontaneously became afebrile and asymptomatic, the chest rales clearing by the end of the first week of observation.

Laboratory data on admission included a white blood cell count of 10,000 per cu. mm. with a normal differential, a sedimentation rate of 26 mm. per hour and a 3-plus CRP. Blood cultures on the third through the sixth day of illness were negative. Streptomycin-resistant P. tularensis was isolated from the pharyngeal washing collected on the third day of illness. A gastric washing obtained on the sixth day of disease was positive (the patient was afebrile and asymptomatic). Pharyngeal and gastric washings were negative on the sixteenth day of illness.

Chest films on admission revealed a prominent left hilum and a juxtahilar, 4 by 5 cm., bronchopneumonic patch located in the upper lobe of the left lung. These abnormalities regressed within the first week and thereafter slowly cleared, being no longer evident a month after onset.

Because of the prompt remission of symptoms no specific therapy was instituted. The CRP was negative by the thirteenth day while the sedimentation rate reached normal by the thirty-ninth day of illness. The agglutinin titer rose to 1:1280 and 1:2560 on the sixth and ninth days of illness, respectively; one year later it was still 1:640. The reaction to the skin test was positive on the eighth day of illness. Throughout a twelve-month follow-up he has remained well.

Comment: This case demonstrates how minimal and self-limited the symptoms may be in laboratory-acquired tularemia. The hilar lymph node enlargement and juxtahilar bronchopneumonia occurred in the absence of severe illness or respiratory symptoms and the positive reaction to the skin test was helpful in suggesting the diagnosis. P. tularensis was isolated from pharyngeal and gastric washings during the first week of disease. Such a mild illness was noted in the eight non-hospitalized patients and one other ward patient. No correlation between the number of previous tularemia vaccinations and severity of disease was evident. This patient exemplified another unusual feature: a fourfold rise in tularemia agglutinin titer appeared in the first week of illness; such an early rise was observed in only one other equally mild case.

Case 15 (Fig. 6). This forty-three year old Negro man was admitted on February 16, 1958. He had received an initial tularemia vaccine series seven years earlier; the last of three subsequent boosters had been given approximately four years prior to illness. In October 1957, the agglutinin titer was 1:10. There had been no recognized exposure.

Five days prior to admission the patient noted the sudden onset of chilliness, generalized aching, nasal stuffiness and retrobulbar pain. In the out-patient department three hours later the physical examination was within normal limits with the exception of an oral temperature of 99.6°F. The white blood cell count was 14,900 per cu. mm. with 85 per cent poly-

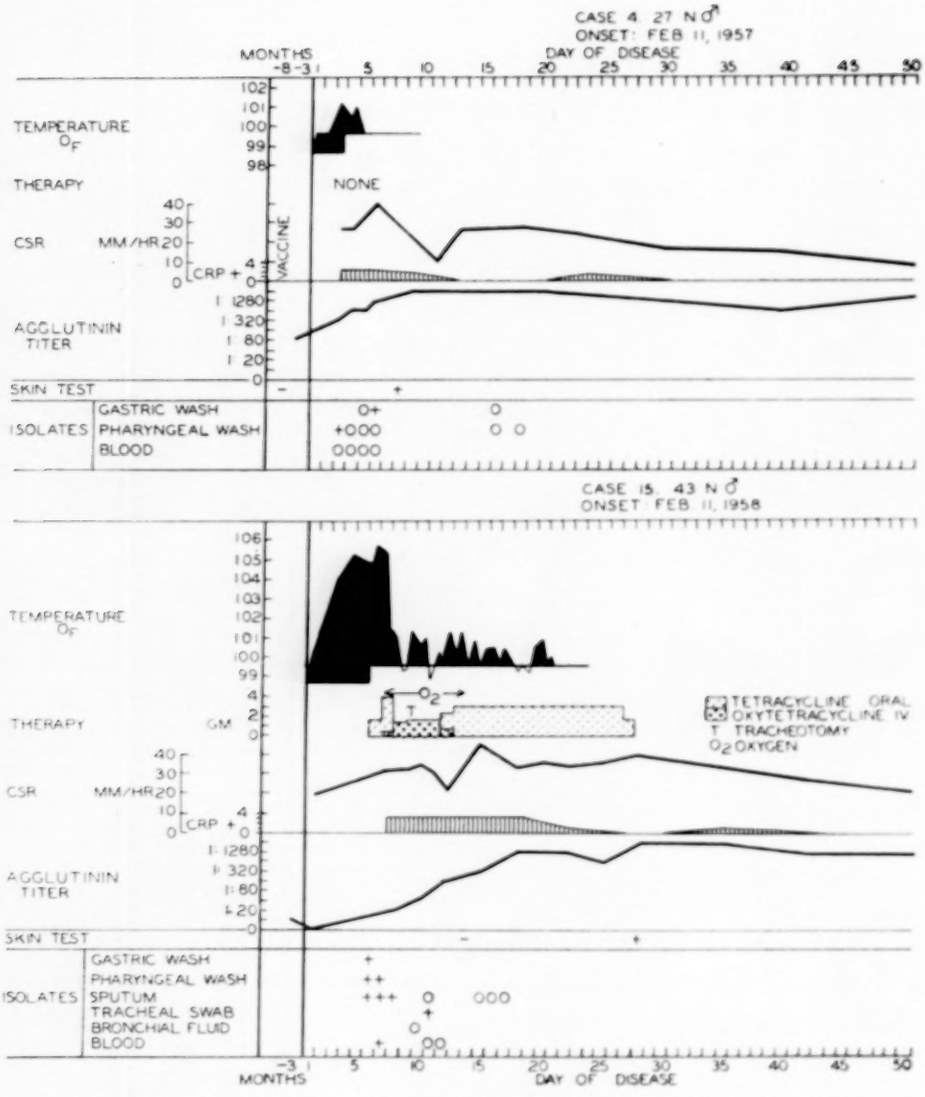

FIG. 6. Summaries of two patients with typhoidal-pulmonic tularemia: mildly ill (Case 4) and severely ill (Case 15).

morphonuclear leukocytes, the erythrocyte sedimentation rate was 19 mm. per hour and the agglutinin titer was negative. Symptomatic therapy was prescribed and the patient was advised to return should the symptoms persist or progress. For the next five days there were severe sweats, fever, sore throat, productive cough, nausea, vomiting, fatigue and weakness which confined him to bed. Dyspnea, blood tinged sputum and moderate diarrhea were now present; occasional oral temperatures varied between 104° and 106°F.

He again sought medical attention on the evening of the sixth day of illness. Physical examination revealed a well developed, well nourished, oriented man who was apprehensive, dyspneic and acutely ill. Rectal temperature was 105°F., pulse 130, regular and full, and the blood pressure 130/70 mm. Hg. There was moderate edema of the nasal mucosa and diffuse hyperemia of the pharynx. Dullness to percussion, crepitant rales and a pleural friction rub were apparent over the upper two-thirds of the left side of the chest but the right side of the chest was clear. The abdomen was not distended, no masses were palpable and peristalsis was normal to auscultation.

On admission laboratory data showed a white blood cell count of 8,800 per cu. mm. with 87 per cent polymorphonuclear leukocytes, of which 70 per cent were bands, a sedimentation rate of 32 mm. per hour, and a CRP of 4 plus. Urinalysis was normal except for 2-plus albuminuria. A sickle cell preparation was negative. The chest film revealed a diffuse, mottled infiltrate in the upper lobe of the left lung, a left hilar prominence and an early reticulated infiltrate in the right cardiophrenic angle. (Fig. 2B.) Lateral films of the chest revealed the apical-posterior segment

of the upper lobe of the left lung to be principally involved.

The severe respiratory illness, normal white blood count and pulmonary infiltrates with hilar adenopathy prompted the diagnosis of "probable tularemia." One hour after admission a 1 gm. "loading dose" of tetracycline was given orally, followed by 0.5 gm. every six hours. Within twenty-four hours the peak rectal temperature of 106°F. had fallen to 101.8°F. However, during this period the respiratory rate rose to 60 with accompanying cyanosis and tracheal rattle. The cough was slightly productive; there were wet rales throughout both lung fields indicating an extension of the disease process. Chest films on the seventh day of illness revealed multiple 0.5 cm. lesions throughout the right and left lung fields. Because of abdominal distention and severe diarrhea, the oral administration of tetracycline was discontinued and oxytetracycline, 1.5 gm. daily, was given intravenously for the next five days. Supportive therapy consisted of intravenous fluids and electrolytes, oxygen by tent, and intermittent nebulization of a bronchodilator along with intravenously administered aminophylline in an attempt to decrease the respiratory distress. Despite control of the fever and absence of vascular collapse, the patient's condition remained critical. On the eighth day paraldehyde was necessary to control delirium. At this time the shallow and rapid respirations had increased to 70 and the heart rate to 150; the cough was still ineffective, cyanosis continued and the bronchial secretions could not be aspirated by suction.

A tracheotomy was performed on the fourth hospital day to reduce dead air space and to permit more direct aspiration and intermittent removal of the thick, tenacious, yellow, bloody secretions. Within the next forty-eight hours the respiratory rate decreased to 40, the tracheal rattle cleared, and the rales throughout both lung fields decreased. By the sixth hospital day intravenous fluid was no longer necessary; oral administration of tetracycline, 3 gm. daily, was begun and continued for the remainder of a twenty-one-day course of therapy. By the thirteenth hospital day the patient was afebrile, the respiratory rate was normal, and there was only a slightly productive cough. The following day he complained of mild headache and low backache; the rectal temperature was 101.6°F. Because of the presence of several influenza cases on the ward, a pharyngeal washing was obtained and Type-A-Japan-57 was recovered; it was not isolated from earlier pharyngeal washings. Within three days the symptoms subsided. At the completion of therapy the patient's only complaints were moderate fatigue, slight dyspnea on exertion, and mild edema of the right ankle, resulting from chemical phlebitis following intravenous therapy.

Streptomycin-resistant P. tularensis was readily isolated from the sputum on the first three days of therapy. A blood culture on the second hospital day was also positive, as were the gastric and pharyngeal washings. Material taken directly from the trachea on the sixth day of therapy yielded the organism. Sputum specimens during the second week of treatment were consistently negative. A reaction to the skin test was negative on the fourteenth day of illness and positive on the twenty-eighth day. The agglutinin titer rose during the second week of illness to 1:160 and reached 1:1280 and 1:2560 by the third and fourth weeks.

During the second month of convalescence the patient's strength returned, the exertional dyspnea subsided and he regained the 25 pounds lost during the acute phase of illness. The CRP was negative by the twenty-fifth day of illness but the sedimentation rate remained elevated for an additional month. The year's follow-up serial chest films revealed gradual but complete clearing of the linear infiltrate in the area of previous pneumonitis.

Comment: These first two cases represent the extremes of the disease spectrum. The severe grippal and respiratory symptoms, delirium, abdominal distention and diarrhea are characteristic of the fatal forms of untreated, naturally occurring typhoidal tularemia. Isolation of P. tularensis from the blood was consistent with experience in the naturally occurring disease, i.e., positive blood cultures have been obtained usually in the fulminating or terminal phase of illness.

Broad spectrum therapy promptly controlled fever; however, the extensive pulmonary involvement with resulting respiratory insufficiency remained a threat to the patient's survival. The tracheotomy on the fourth day of hospitalization appeared to "turn the tide." The pulmonary infiltrate regressed within the first week of therapy and thereafter slowly cleared. The ability to isolate the organisms regularly throughout the first week of bacteriostatic therapy was a common experience. The skin test was not helpful in early diagnosis since the result was negative during the first two weeks of illness and as usual the rise in agglutinin titer did not occur until later.

Case 26 (Fig. 4). This twenty-two year old white man was admitted to the hospital on August 2, 1957. On the previous day he noted a severe pounding, bilateral, temporal headache, with anorexia and insomnia. The following day nasal stuffiness, moderate sore throat, severe myalgias of the low back and calf muscles, sweats, fever and weakness compelled him to remain in bed. These symptoms appeared three days after a recognized laboratory accident. A co-worker

became ill five days after this exposure with typhoidal tularemia.

The patient had received an initial vaccine series approximately five months prior to the onset of illness; two weeks before infection the agglutinin titer was 1:20.

Physical examination revealed a young white man who appeared moderately ill. The physical findings were limited to a rectal temperature of 103.6°F., pulse 100, and injected pharynx and nasal mucosa.

On admission laboratory data included a white blood cell count of 7,800 per cu. mm., 74 per cent neutrophils, sedimentation rate of 4 mm. per hour and a 4-plus CRP. The following day the sedimentation rate had risen to 30 mm. per hour.

Serial chest films throughout the two-week period of hospitalization were within normal limits. The reaction to the skin test was negative as late as the thirteenth day of illness, but was positive when next tested on the thirty-third day. Streptomycin-resistant P. tularensis was isolated from gastric washings of the second and fourth hospital days, and from pharyngeal washings of the second through fourth days. On the second hospital day, and without specific therapy, the temperature spontaneously fell to near normal only to rise to 103°F. on the following day.

Tetracycline therapy, 2 gm. daily given orally, was started on the fifth hospital day and continued for ten days. Within twenty-four hours the patient was afebrile, the severe grippal symptoms had subsided, and after five days the nasal stuffiness and moderate sore throat had cleared. At the completion of therapy the CRP was normal and the sedimentation rate was falling. It was possible to isolate organisms from pharyngeal washings throughout the first five days of therapy and from gastric aspirates on the third and sixth days of therapy; no further gastric specimens were obtained. Two other specimens were positive, a bronchial lavage on the first day and a nasopharyngeal swab on the second day of treatment.

Seven days after completion of therapy and while on convalescent leave the patient noted the return of general malaise, mild sore throat and feverishness. He was readmitted on the seventh day of relapse. Physical examination revealed a mildly ill person with tenderness over the anterior, cervical and submandibular chains of lymph nodes and a granular, inflamed, posterior pharynx. The white blood cell count was 12,700 per cu. mm., with 63 per cent neutrophils and 31 per cent lymphocytes of which 50 per cent were atypical. The sedimentation rate was 36 mm. per hour, and CRP 4-plus. Serial chest films were within normal limits.

The differential diagnosis rested principally between a relapse of tularemia and infectious mononucleosis. Results of subsequent serial heterophil tests were normal; numerous pharyngeal washings and one gastric aspirate were again positive for streptomycin-resistant P. tularensis. The report of these positive isolations prompted a second two-week course of tetracycline therapy (2 gm. daily given orally). Symptoms subsided by the fifth day of treatment; nevertheless it was possible to isolate the organism from the pharyngeal washings as late as the sixth day of therapy. At the completion of therapy the CRP had again returned to normal but the sedimentation rate was still 28 mm. per hour. The sedimentation rate was within the normal range by the fifth month.

The agglutinin titer remained at a 1:40 level throughout the second week of illness but by the twenty-fifth day, three days after relapse, it was 1:640 and reached the peak level of 1:1280 by the thirty-third day. During one year's follow-up the sedimentation rate, CRP, chest film and physical examination have remained within normal limits. However, the patient has complained of mild generalized muscular and joint aches on damp days, effectively controlled with aspirin.

Comment: This is a case of moderately severe tularemia in which serial chest films were within normal limits. The patient and his associate became ill on the third and fifth days following an accident which could have been expected to create an aerosol containing P. tularensis. In addition to the severe grippal symptoms, nasal stuffiness and sore throat were prominent complaints. In our patients these symptoms were common and occurred with or without roentgenographic evidence of pulmonary changes. The injected and slightly granular appearance of the posterior pharynx was noted in approximately half of the patients. The finding of 5 to 15 per cent atypical lymphocytes during the acute phase of tularemia was not uncommon. There were the usual prompt responses to the administration of tetracycline during the initial and relapse episodes, yet it was possible to isolate the organism on both occasions through the sixth day of therapy.

Of the five relapses observed in this series of patients, all had in common the following: return of symptoms seven to fourteen days after completion of at least one week of tetracycline therapy started during the first week of disease, milder symptoms than initially, and a prompt response when retreated with tetracycline. Four of these five patients initially had pulmonary lesions, three of which worsened during relapse but regressed promptly with therapy.

CASE 22. This twenty-five year old Negro man was admitted on October 16, 1956. Eight days before admission he noted night sweats, evening fever, moderate anorexia and mild frontal headache. Al-

though the symptoms did not increase in severity and he continued to work daily, their persistence prompted his admission.

He had received an initial vaccine series almost four years earlier; there were no intervening boosters. The agglutinin titer three months before infection was 1:40. There had been no recognized exposure but he had worked with P. tularensis on a single occasion four days prior to onset of symptoms.

Physical examination was non-contributory; the oral temperature was 100.4°F. Laboratory data on admission showed a white blood count of 8,520 per cu. mm. with normal differential, a sedimentation rate of 26 mm. per hour and a CRP of 3-plus. The chest film did not demonstrate any abnormalities. During the first three days of observation the patient complained of moderate night sweats, anorexia, slight frontal headache and low back ache. The oral temperature ranged between 101° and 102°F. On the fourth hospital day, and twelfth day of illness, a painless swelling developed just anterior and overlying the angle of the right mandible. This mass measured 4 by 4 cm.; it was round, firm, non-tender and seemingly fixed to the mandible. There was an associated 1 cm., firm, non-tender, easily movable submental node. Dental and mandible films as well as daily examination of the oral cavity did not reveal any abnormality.

Therapy with chloromycetin, 2 gm. daily given orally, was started on the fourth hospital day and continued for ten days. Within forty-eight hours the mild symptoms subsided and the temperature fell to 99° to 100.0°F. where it persisted for the next six weeks. The mass on the right mandible remained unchanged and the elevated sedimentation rate and CRP persisted in spite of therapy.

Pharyngeal washings on the first and second hospital day were negative for P. tularensis but a gastric washing obtained on the third hospital day showed streptomycin-resistant organisms. The result of the skin test on admission was positive and the agglutinin titer rose to 1:640 by the seventh and 1:1280 by the eleventh hospital day.

The mass remained unchanged and the patient was transferred to another hospital for an excisional biopsy on the thirty-sixth day of illness. At surgery the mass appeared to be lymphoid tissue, firmly adherent to the adjacent tissue but not involving the bone. It was removed by curettage. The biopsy site rapidly healed. No organisms were cultured from the biopsy specimens; microscopic examination disclosed areas of necrosis surrounded by epithelioid cells and a few giant cells; there were numerous infiltrating round cells, plasma cells and macrophages. A diagnosis of an inflammatory granulomatous reaction compatible with tularemia was made.

Following surgery a low grade evening fever persisted; a ten-day course of therapy with orally administered novobiocin, 2 gm. daily, was started on the sixth day after surgery with the temperature subsiding to normal within twenty-four hours. However, on the seventh day of therapy moderate fever and a pruritic, erythematous, macular rash developed which cleared three days after discontinuing the antibiotic. One month later the sedimentation rate and CRP were normal. The patient has remained well throughout a one year follow-up period. The result of the skin test has remained positive and the agglutinin titer at one year was 1:320.

Comment: The history of potential exposure to the organism permitted an estimation of the incubation period. The absence of any respiratory symptoms was noted in only four of the forty-two cases. Nevertheless, the organism was obtained from gastric washings. The mild but persistent symptoms were consistent with the type of illness seen in many of our patients.

The appearance of the lymphoid mass over the right mandible on the twelfth day of illness was an unusual feature. Chloromycetin promptly controlled the patient's symptoms but the low grade fever and mass persisted. This has been noted also following the late use of antibiotics in naturally occurring ulceroglandular disease. Failure to isolate the organism from the involved node in this patient was to be expected; even without treatment the recovery of P. tularensis from local nodes is rare after the first month of illness.

More commonly, cervical lymph node involvement is associated with the oropharyngeal form of disease. In this case no oral lesion was apparent, nevertheless direct extension from the oral cavity seems probable.

Case 11. This thirty-four year old white man was first admitted on February 5, 1956. Four days earlier he noted the onset of generalized malaise, frontal headache, raw throat, nasal stuffiness, dry nonproductive cough, intermittent shaking chills, drenching sweats and feverishness. He had received an initial vaccine series fourteen months prior to his infection, with one intervening booster six months before.

Physical examination revealed a moderately ill, toxic appearing man with an oral temperature of 103°F.; there were diminished breath sounds and inspiratory rhonchi over the posterior lower left lung field.

On admission laboratory studies revealed a white blood cell count of 10,300 per cu. mm. with a normal differential and sedimentation rate. A chest roentgenogram showed a 3 by 2 cm., oval density merging with the hilum in the third anterior interspace of the left lung.

Blood specimens collected on the second through fourth hospital days were negative for P. tularensis. Pharyngeal washings of the sixth through eighth days of illness produced streptomycin-resistant P. tularensis.

Therapy was instituted on the second hospital day (sixth day of illness) and consisted of 2 gm. of tetracycline administered orally and 1 gm. streptomycin administered intramuscularly, daily for eleven days. During the first forty-eight hours of treatment the oral temperature fluctuated between 100° and 105°F.; there were alternating severe chills and sweats. At each temperature spike aspirin was given. After seventy-two hours the patient was afebrile and essentially asymptomatic with the exception of dull pain in the left side of his chest and a mild non-productive cough; these symptoms had subsided by the completion of therapy. The pulmonary lesions regressed and there was only a slight infiltrate by the twenty-fourth day of illness. No further films were obtained until nine months later; at this time there was no residual. The tularemia agglutinin titer twenty-seven days prior to illness was 1:640 and varied between this level and 1:1280 throughout the illness. No skin test was attempted during the illness; a year later the reaction was positive and the agglutinin titer was 1:160.

The patient enjoyed good health until shortly before his second admission on December 31, 1957. Seven days earlier he had noted intermittent feverishness, chilliness, night sweats, increasing fatigue, generalized myalgia, mild frontal headache, non-productive cough and diffuse substernal chest soreness. Physical examination revealed a rectal temperature of 101°F. and a few crepitant rales in the left mid-axillary line at the level of the angle of the scapula.

On admission laboratory data showed a white blood cell count of 13,000 per cu. mm. with a normal differential, a sedimentation rate of 26 mm. per hour and a CRP of 4-plus. A chest film demonstrated a 2 cm. oval infiltrate with indistinct borders lying in the mid-portion of the lower left lung field, slightly lateral to the previous area of involvement. From the gastric washing obtained on admission streptomycin-sensitive P. tularensis was obtained. The organism was also isolated from a small amount of sputum obtained on the second and third hospital days. Frequent pharyngeal washings were consistently negative.

The oral administration of tetracycline, 2 gm. daily, was started on the second hospital day and continued for ten days. The grippal symptoms and fever subsided within forty-eight hours; the dry cough and vague chest distress three days later. Sedimentation rate and CRP were normal by the twenty-eighth day of the disease; the pulmonary infiltrate had cleared by the forty-second day. During the second through the sixth weeks of illness the titer fluctuated between 1:320 and 1:640 and reached a peak of 1:1280 on the seventieth day. Throughout a twelve-month follow-up the patient remained well.

Comment: During both illnesses, upper and lower respiratory tract symptoms were present; and bronchopneumonia involved the lower lobe of the left lung. During the first episode the symptoms were moderately severe and incapacitating. It is probable that the use of aspirin at the peaks of fever contributed to the disability, by producing wide swings in the temperature with alternating severe chills and sweats. This has not been observed since the use of antipyretics was discontinued. This was the only instance of recognized reinfection. The initial disease was caused by a streptomycin-resistant organism whereas reinfection was caused by a streptomycin-sensitive strain; both illnesses occurred with high initial agglutinin titers.

COMMENTS

The American literature clearly describes the etiology, epidemiology, clinical course and pathology of naturally occurring tularemia. In this country, as a result of contact with lower animals or of insect bites, approximately 90 per cent of such cases are of the ulceroglandular variety. In contrast, laboratory-acquired disease is principally of the typhoidal form.

In laboratory infections circumstantial evidence points to the respiratory tract as the principal portal of entry. The potential exposure to air-borne P. tularensis is a constant hazard. The appearance of illness in the absence of a recognized accident and the occasional case following remote contact implicate this route of infection and suggest a low infective dose. There has been no reported man-to-man transmission of tularemia. No unusual precautions were taken in the care of these patients or in obtaining various respiratory secretions from which the organisms were commonly isolated. There were no secondary cases of tularemia among hospital personnel.

In keeping with the previously reported typhoidal cases the initial respiratory symptoms were coryza, pharyngitis, substernal tightness and cough at the onset of illness, without regard to findings on chest roentgenograms. It is apparent that the conventional disease classification of typhoidal versus typhoidal with pulmonary involvement does not connote a difference in pathogenesis but merely the extent of respiratory involvement.

Before any effort can be made to generalize

from these cases it is essential to examine the possible influence of prior vaccination. There are now over 200 reported laboratory infections in vaccinated personnel. Phenolized and acetone-extracted vaccines do not protect white mice or monkeys from subsequent infection with minimal doses of a virulent strain. Foshay et al. [*4*] in naturally occurring tularemia, and Kadull et al. [*5*] and Van Metre and Kadull [*1*] in laboratory-acquired untreated tularemia have reported apparent modifications of severity and of duration of disease in vaccinated personnel. Because of the unknown size of the control group and lack of data on the relative risk of exposure it was not possible to evaluate the vaccine as a disease preventive. These authors describe vaccinated persons with unexplained rises in agglutinin titers. Whether such cases were truly subclinical or similar to the mildly ill, untreated patients of our series is a moot question. Asymptomatic infection in the unvaccinated person has not been documented with the virulent strain common to North America.

Recently Saslaw et al. [*7*] have defined the role of a phenolized vaccine in a controlled volunteer study. A challenge of approximately ten organisms of a fully virulent strain of P. tularensis via the intracutaneous route was employed in three groups of men: (1) non-vaccinated, (2) vaccinated and challenged three weeks later, and (3) vaccinated, followed by a booster six months later, and challenged three weeks after the booster. Local lesions developed in all challenged subjects. Eleven of twelve non-vaccinated men had associated fever and constitutional symptoms, while only three of fourteen vaccinated men and two of five vaccinated-plus-booster men were symptomatic. Infection was not prevented; prior vaccination did modify the severity of the disease resulting from a small intracutaneous challenge.

In contrast, this same group has reported respiratory challenge of men with a dose of 25 to 50 organisms. This produced overt illness in six of eight control subjects and a similar initial clinical picture in eight of fourteen men previously immunized with a phenolized vaccine. The incubation periods were identical, as were the symptoms manifested during the initial hours of fever. The evidence from all sources is thus rather convincing that killed vaccines do not prevent initiation of disease nor is the severity of the clinical picture in such vaccinated subjects necessarily altered.

Killed vaccine preparations are reported to be ineffective in the prevention of tularemia in Russia [*8*], even though the Russian strains of P. tularensis are considered by them to be less virulent than American strains [*9*]. In consonance with these laboratory data are clinical observations of an over-all case fatality rate of less than 1 per cent, a milder illness and the occurrence of subclinical infections. For the control of tularemia in the Soviet Union, live attenuated P. tularensis vaccines have been used extensively [*8*]. Derivatives of a Soviet strain have been studied in America and vaccination of men has been shown to offer significant degrees of protection against large respiratory challenges with a fully virulent American strain [*10*].

In our experience typhoidal tularemia in American laboratory workers receiving killed P. tularensis vaccine differs in severity from the reported naturally occurring disease. However, the causative strains, conditions under which exposure occurred, time of onset of therapy and type of therapy are not necessarily comparable. Perhaps, too, the high index of suspicion as well as the system of medical investigation employed in the present study permitted an appreciation of the wide range of severity of the typhoidal form of disease. Only 19 per cent of our group of patients were considered to be severely ill in spite of the fact that more than half of the hospitalized patients were admitted after the first week of illness. Of the eight severely ill patients, half were Negroes, an incidence of 44 per cent of this racial group, as opposed to 16 per cent among white patients. No relationship could be established between the severity of illness on admission and the number of booster series or the time interval from the last booster. A person who had received his initial series four years earlier with no subsequent booster was as likely to have mild disease as a person who had received his booster within the last six months.

Despite the wide range of severity of illness the chest films on admission revealed abnormalities in twenty of thirty-four typhoidal cases. This 59 per cent incidence of pulmonary involvement compares favorably with the reported 50 to 77 per cent in naturally occurring typhoidal tularemia. In sixteen cases, eight of which were not ulceroglandular Ivie [*11*] noted an oval area of infiltration in nine and hilar adenopathy in five. In ten of the cases there was some degree of pleural effusion which usually occurred late in the course of illness. Dennis and Boudreau [*12*]

reported fourteen cases of pleuropulmonary tularemia, seven of which were not ulceroglandular in origin. In addition to the findings of Ivie, these observers, emphasized the frequently widespread, bilateral pulmonary involvement. Of our seventeen patients with pneumonia, fifteen had oval 2 to 8 cm. infiltrates (twelve single and three multiple), one had an irregularly shaped, diffuse bronchopneumonic patch, and one had lobar consolidation. There was attending hilar adenopathy in eight and a pleural effusion in two of these patients. Another patient had hilar adenopathy, perihilar streaking and pleural effusion. Pleural effusion was observed as the only abnormality in two cases. The presence of an oval pneumonic patch or pleural effusion associated with hilar adenopathy occurring during a febrile illness should strongly suggest the possibility of tularemia [*13*].

Because of the danger of laboratory infections, few hospital laboratories have made serious efforts to recover the organism in typhoidal tularemia. In endemic areas the conventional approach has been to consider the possibility of typhoidal tularemia in any severe atypical pneumonia or febrile illness not responding to penicillin therapy. In such instances a two- to three-day course of streptomycin results in prompt clinical improvement and has been recommended as a therapeutic test, the diagnosis being confirmed by the appearance of agglutinating antibodies during the second week of illness with a subsequent rise between the fourth and sixth weeks [*14*]. Obviously such an approach was not applicable under the circumstances described in this paper.

As already noted the usual diagnostic aids were of little specific value early in the course of the disease. The necessity to establish an early definitive diagnosis prompted efforts to isolate the organism. Examination of gastric and pharyngeal washings, and sputum when available, yielded the organism in twenty-eight of thirty-three typhoidal cases as late as the third week of untreated illness. Twenty-two of these isolates were resistant to streptomycin, seven were sensitive (Case 45: both sensitive and resistant organisms were isolated). Sputum samples and gastric washings were superior to pharyngeal washings for recovery of P. tularensis. In these cases in which the culture was negative, but guinea pig inoculation was positive, specific identification was not usually possible until the fifth day following admission (range four to seven days). When the organism could be cultured, a definitive diagnosis was made within forty-eight to seventy-two hours.

Early in the study the ratio of positive cultures to positive animal inoculations was low. With the simple maneuver of using the same quantity of inoculum as given to animals on multiple culture plates, the results became almost identical for streptomycin-resistant organisms. For streptomycin-sensitive organisms, limited experience with a crystal violet-penicillin modification of GCB media has indicated that cultural procedures will most probably give equally satisfactory results. In one instance the use of the two selective media results in the isolation of streptomycin-resistant and streptomycin-sensitive organisms of P. tularensis from a patient who had been exposed to both. Only a streptomycin-resistant strain was isolated in guinea pigs. Various methods of shortening the time required for a laboratory diagnosis are under study and will be reported elsewhere [*15*].

It is well known that P. tularensis can be isolated from the sputum or pleural fluid of patients with severe pulmonary involvement. In untreated disease the organism has been isolated from the sputum of a pneumonic patient as long as forty-nine days and from pleural fluid as late as four months. It has not been generally appreciated that the organism can be isolated from sputum samples of patients without abnormal findings on chest roentgenograms. Johnson [*16*] and Larson [*17*] each describe a single case of typhoidal disease in children without demonstrable roentgenographic pulmonary lesions in which organisms were isolated from sputum by mouse inoculation during the third week of illness. Gastric and pharyngeal washings have not been successfully exploited previously as sources of diagnostic material. These specimens also may yield positive cultures whether chest x-ray evidence of pulmonary involvement is present or not.

In vitro studies demonstrate that streptomycin is bacteriostatic for most strains of P. tularensis at a concentration of less than 0.4 μg. per ml. and bactericidal at 1.9 μg. per ml. The resistant strains isolated from our patients were not sensitive to 10 or 100 μg. per ml. In contrast, the broad spectrum antibiotics *in vitro* are bacteriostatic for P. tularensis. Growth of the isolates from each of our patients was inhibited by concentrations of 1 μg. per ml. of tetracycline. This difference in drug action undoubtedly accounts

for the continued isolations of P. tularensis from the nasopharynx of the patients for several days after the initiation of therapy with broad spectrum antibiotics. Similar observations have been made in other diseases.

The work of McCrumb et al. [*18*] in induced ulceroglandular tularemia in volunteer subjects has emphasized the difference between the therapeutic action of streptomycin and of the broad spectrum drug Chloromycetin. These investigators inoculated volunteer subjects intradermally with 400 to 10,000 P. tularensis cells (Rector strain). In the six control subjects reddish papules appeared within twenty-four to forty-eight hours. By the third day after inoculation the local lesions had enlarged from 1 to 2 cm., and moderate axillary adenopathy, fever and mild constitutional symptoms were present. At this point the disease was promptly controlled with five days of therapy with either Chloromycetin or streptomycin. Daily therapy in four patients consisted of 2 gm. of streptomycin and of 3 gm. of chloromycetin in two patients. Those who received Chloromycetin experienced febrile relapses whereas the streptomycin-treated patients remained well. These observations are in agreement with the results following streptomycin or broad spectrum drug administration in naturally occurring tularemia. Unlike brucellosis, there is no requirement for both streptomycin and broad spectrum drug therapy.

The generally recommended dosage of streptomycin or dihydrostreptomycin is 0.5 to 2 gm. daily for five to ten days depending upon the severity of illness. Relapses have not been reported following such therapy. The reported clinical experience with the tetracyclines and Chloromycetin has been less extensive. Their effectiveness in controlling the acute phase of illness has been comparable to that of streptomycin, i.e., precipitous drop in fever and control of symptoms within twenty-four to forty-eight hours. Corwin and Stubbs [*19*] pointed out that chlortetracycline was effective in securing remissions but, unlike streptomycin, was not curative. They described eight patients who completed a dose schedule ranging from 8 gm. in four days to 12 gm. in six days. Seven returned to the hospital within five to nine days with exacerbation of symptoms. The day of disease on which therapy was started is not indicated. Parker et al. [*20*] observed two relapses among six patients treated with Chloromycetin. The therapy schedule consisted of "loading doses" of 2 to 3.5 gm. and 2 to 3 gm. daily thereafter. One of the patients was a laboratory worker who had the typhoidal form of disease with pulmonary involvement. Therapy was instituted on the sixth day of illness starting with a 3 gm. "loading dose" and 3 gm. daily for 5.3 days; fever was promptly controlled. Four days after completion of therapy, fever and symptoms returned. A 10 gm. course of Chloromycetin promptly controlled the symptoms but two days after completion of this second course, the symptoms returned. A third course of 18 gm. for seven days brought the illness under control.

In our patients the rapid decline in fever, control of symptoms within twenty-four to forty-eight hours, and prompt regression of abnormal sedimentation rate, CRP and chest roentgenogram after the institution of broad spectrum drug therapy were markedly uniform. The response was equally striking in the more serious cases. However, five relapses were noted among the fifteen patients treated within the first week of illness. Such relapses were milder than the initial disease and promptly responded to readministration of the same drug. Reappearance of overt disease was not observed among the sixteen patients treated after the first week of infection who received antibiotics for a comparable period. Nevertheless, four of sixteen patients so treated had a subclinical rebound (rise in sedimentation rate) which occurred several days after cessation of drug.

The broad spectrum drugs fail to free the host of P. tularensis. If therapy is started during the initial week of illness, before there is a period of adequate antigenic stimulus, relapses may occur after completion of seven to ten days of treatment; a higher relapse rate may be expected if less than seven days of broad spectrum therapy is given. However, after approximately a week of untreated illness (or, in other words, antigenic experience) the host's immune mechanism is presumably sufficiently stimulated to deal with the renewed bacterial growth which occurs when the seven- to ten-day course of bacteriostatic drug therapy is completed. Time of initiation of therapy probably is not the sole factor, since patients treated in the second or third week of illness usually had milder presenting symptoms. As yet there is not a proved therapeutic regimen which will completely prevent relapses if the administration of broad spectrum antibiotics is begun during the first week of illness. It is possi-

ble that, as in scrub typhus, relapses can be prevented by early but interrupted therapy. The problem is now under study in animals and man.

The occurrence of relapses when broad spectrum drugs are used for therapy strongly implies that they would not be particularly effective as a prophylactic measure. McCrumb et al. [*18*] have clearly shown this in the experimental ulceroglandular disease. Seventeen volunteer subjects were challenged and one hour later antibiotic therapy was started. In nine of the ten patients who received streptomycin for five days disease or agglutinating antibodies failed to develop. Overt disease followed the cessation of therapy in only one subject, a 200-pound man who received a total of 3 gm. of streptomycin over a five-day period. In contrast, a five-day course of Chloromycetin therapy failed to prevent disease in five of seven patients but did prolong the incubation period; ulceroglandular disease then developed which was responsive to a second course of this antibiotic.

It is widely accepted that one attack of tularemia confers permanent immunity. However, there are at least eight well documented reinfection ulceroglandular cases in this country. Taylor [*21*] described two patients with naturally occurring ulceroglandular tularemia who returned, one month and eighteen months later, respectively, with second distinct, acute attacks of ulceroglandular tularemia. Both of these patients had been treated with antibiotics within the first week of initial illness. Green and Eigelsbach [*22*] and Francis [*23*] reported on the remaining six patients with reinfections; they were laboratory-acquired, mild ulceroglandular disease which appeared from a few months to as long as several years after their initial untreated illness. One reinfection (Case 11) occurred among our forty-two patients. On both occasions this patient had bacteriologically-proved typhoidal disease with pulmonary involvement. In a previously cited investigation [*7*] on ulceroglandular disease, eight volunteer subjects were rechallenged six months after an initial illness. These subjects had received streptomycin therapy and agglutinins had developed in all. Following the second challenge local lesions developed in all patients, but only two had constitutional symptoms. These observations suggest that mild reinfections of ulceroglandular or typhoidal disease may occur more often than is appreciated in endemic areas. How often "winter" colds or slowly healing cuts in rabbit hunters represent reinfection is unknown. Following re-exposure, and depending upon the interplay between the host's immunity, challenge dosage and virulence of the organism, illness in some form may occur.

Finally, there is the problem of "chronic tularemia." Even the extensive pulmonary lesions begin to resolve after one to two weeks of appropriate antibiotic therapy and completely clear within a few months. The exception is the occasional instance of residual pleural thickening and this was not seen with early and adequate therapy. None of our patients had residual respiratory symptoms or roentgenologic evidence of permanent pulmonary lesions. Of our forty-two patients, only two complained of persistent mild malaise; both followed relapses. One became asymptomatic within six months; the other continued to complain during a year's follow-up of diffuse arthralgia accentuated by damp weather. These patients were followed up by one group of physicians who, from the onset of the study, made every effort to control the iatrogenic factors to the benefit of the patient. It would appear that the diagnosis of "chronic tularemia" has often been a convenient wastebasket category for any unexplained symptoms following typhoidal tularemia. Latent psychoneurotic symptoms can be brought on by acute illness in patients who are less stable emotionally. Physicians must recognize that these are symptoms of convalescence and not of active infection. Appropriate reassurance is the crucial therapy.

SUMMARY

Forty-one vaccinated and one unvaccinated laboratory personnel acquired typhoidal tularemia as a result of probable aerosol exposure. The resulting disease was characterized by nonspecific grippal and respiratory symptoms of varying severity, the majority being mildly to moderately ill. Phenolized and acetone-extracted vaccines were ineffective in preventing clinical disease but probably modified the course of illness.

Among the thirty-four hospitalized patients, twenty had abnormal findings on chest roentgenograms. Fifteen had oval, bronchopneumonic lesions, one had diffuse bronchopneumonia, one had lobar pneumonia. Pleural effusion occurred in five patients, in two as an isolated finding. Hilar adenopathy occurred in nine patients. Such findings should prompt

consideration of tularemia in the differential diagnosis.

Agglutinin and hemagglutinin serum levels were not useful in the early recognition of disease. Results of skin tests were positive in seventeen of thirty-one patients when first seen; however, this was useful in suggesting the diagnosis in only thirteen since four were known to have had positive reactions to skin tests prior to onset of illness. P. tularensis was regularly isolated during the first few days of hospitalization from sputum when available (fourteen of sixteen patients), and frequently from gastric aspirates (twenty-two of thirty-one patients) and pharyngeal washings (eighteen of thirty-two patients), and as late as the third week of untreated illness by guinea pig inoculation or plating on appropriate culture media.

The illness of approximately 75 per cent of the hospitalized patients resulted from laboratory strains resistant to streptomycin, the remainder from streptomycin-sensitive strains. Two of the hospitalized and seven of the non-hospitalized patients with minimal symptoms were untreated. Thirty-one of the hospitalized group were treated with broad spectrum antibiotics given in four equally divided doses; in the majority of patients 2 gm. were given daily for seven to fourteen days. Following initiation of therapy the mean duration of any fever was approximately three days; there was corresponding improvement of symptoms within forty-eight hours and with the exception of the critically ill patient all patients were fully ambulatory within one week. With treatment there was prompt regression of pulmonary lesions and gradual clearing over the following four to six weeks. P. tularensis could be isolated from sputum samples, pharyngeal washings or gastric aspirates throughout the first week of therapy.

Among fifteen of the patients so treated during the first week of disease, five suffered relapses whereas in sixteen patients with similar therapy instituted after the first week of disease no relapses occurred. It is apparent that this group of drugs are bacteriostatic and fail to eradicate the organism from the host. When such therapy is started early in illness the host's immune status often is incapable of preventing relapses. Retreatment during relapse with the same antibiotic is effective.

Mild residual and unexplained symptoms following control of the acute phase of disease were observed in only two patients, a much lower incidence than usually cited. This low occurrence of residual complaints is attributed to the continuing physician-patient relationship with appropriate reassurance during the twelve-month follow-up.

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Case Reports

Medullary Cystic Disease of the Kidney, with Some Observations on Ammonium Excretion*

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Isolated cystic dilatation of the collecting tubules of the kidneys is an uncommon condition. It has been reported in the literature under such descriptive synonyms as medullary sponge kidney [*1*] and cystic disease of the renal pyramids [*2*]. In 1939 Lenarduzzi [*3*] published the first clinical and radiological description of a case, although the condition had been noted much earlier [*4–6*]. Lindvall [*1*] suggested that it is more common than would appear from the few reported cases, having detected thirty-one cases from the urographic records of the Karolinska Sjükhuset over six years. He described the radiological appearances of medullary sponge kidney in detail but not the clinical features. There is little information on disturbances of renal physiology in this condition because most of the patients in whom investigations have been reported were in a state of chronic renal failure, the results consequently being non-specific.

The present case report indicates a possible link between the structural abnormality and the functional disturbances in the kidney. The patient showed an abnormally low excretion of ammonium in the presence of a normal glomerular filtration rate, an unusual observation [*7–9*]. As medullary cystic disease consists of cysts of the collecting tubules [*6,10*], this report provides indirect evidence in support of the contention that the collecting tubules produce ammonia [*11,12*].

CASE REPORT

A forty-one year old man was first admitted to the hospital on December 31, 1953, complaining of shortness of breath. He had been well until two months previously when he noticed gradually increasing breathlessness on exertion and swelling of his right foot during the day. He slept lying flat and was not breathless at rest. He had never experienced chest pain. He suffered from a mild cough which was occasionally productive of whitish sputum, and infrequent attacks of palpitations during which he noted irregularity of the heartbeat. For many years he had passed small quantities of urine one to three times at night but there was no increased frequency of micturition during the day. Other than a mild attack of measles as a child, he had previously been ill only once. This occurred at the age of thirty-two years when he suddenly felt ill, with pain in the back and burning on micturition. He was unaware of any swelling of the face or legs. His temperature was found to be 104°F. and his urine was reddish in colour. "Bright's disease" was diagnosed and he was treated conservatively for four weeks during which time he passed unusually small quantities of urine. He was away from work for two or three months but remained well thereafter for nine years until the onset of the present illness.

Systematic enquiry revealed no other points of significance except that there was no family history of cardiovascular disease and no recurrence of his kidney ailment.

On physical examination the patient appeared well but was found to have slight enlargement of the heart with a left ventricular type of maximum cardiac impulse, a pulsus bigeminus and a soft apieal systolic murmur. The blood pressure was 130/85 mm. Hg. The electrocardiogram showed left axis deviation and bigeminal rhythm due to ventricular extrasystoles.

Radiography of the chest demonstrated left ventricular enlargement and wedging of the sixth dorsal vertebra. An intravenous pyelogram was re-

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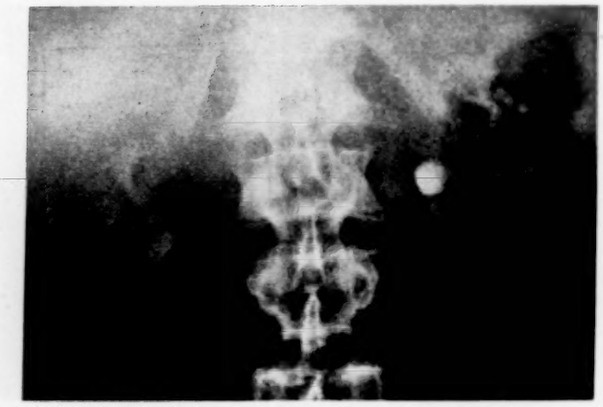

FIG. 1. Intravenous pyelogram at twenty minutes.

ported as being within normal limits, but ankylosis of the sacro-iliac joints was noted (unfortunately the films were not avai able for review).

The urine examination revealed a specific gravity of 1.012 and albumin 4 plus (protein concentration was 80 mg. per 100 ml.). Microscopy of the centrifuged specimen showed occasional red cells and hyaline and granular casts. Total phenolsulphonphthalein excretion was 73 per cent in two hours.

The blood urea was 32 mg. per cent and the average urea clearance was 102 per cent of normal renal function. The plasma uric acid was 3.8 mg. per cent, plasma creatinine 1.2 mg. per cent. The serum albumin was 3.2 and globulin 2.6 gm. per cent. The serum sodium was 126 mEq. per L., serum potassium 4.3 mEq. per L., the serum alkaline phosphatase 5.3 King-Armstrong units (normal 4 to 13 King-Armstrong units) and the acid phosphatase 1.4 units (normal 0.8 to 4.6). C-reactive protein was 2 plus positive. Results of the modified Ide test were negative. The haemoglobin was 20.7 gm. per cent, white blood count 7,200 per cu. mm., with a normal differential and platelet count.

A diagnosis of chronic glomerulonephritis (latent phase) was made and he was treated by rest in bed. He felt better although there was no objective change in his condition.

He was readmitted five and a half years later, again complaining of shortness of breath. This symptom had persisted since his first admission to hospital but had been relatively mild until a year prior to this admission when it gradually increased.

Physical examination revealed enlargement of the heart (heaving maximum cardiac impulse in sixth intercostal space 4 inches from the midline), blood pressure of 180/120 mm. Hg and arteriovenous nicking on funduscopic examination. Scanty creptitations were audible at the bases of both lungs but there was no congestive cardiac failure. The heart rhythm was normal. The electrocardiogram showed left axis deviation and left ventricular hypertrophy. A standardized exercise test showed definite excess ventilation on effort thus confirming the presence of dyspnoea.

The urine contained albumin 4 plus (the daily Esbach tests indicating a proteinuria of 0.5 to 1.5 gm.). Microscopic examination of a centrifuged specimen revealed occasional red blood cells and numerous hyaline and granular casts, but no organisms. Repeated cultures of urine were negative. The blood urea was 28 mg. per cent. Paper electrophoresis of serum proteins revealed albumin 3.3 gm. per cent, alpha-1-globulin 0.4 gm. per cent, alpha-2-globulin 1 gm. per cent, beta-globulin 1.5 gm. per cent, gamma-globulin 1.2 gm. per cent; serum sodium was 145 mEq. per L., serum potassium 4.2 mEq. per L., plasma chloride 98 mEq. per L., plasma CO_2 content 24.5 mEq. per L., and plasma cholesterol 205 mg. per cent. Results of the modified Ide test were negative. A battery of liver function tests revealed no abnormality. Haemoglobin was 19.1 gm. per cent, white blood count 10,900 per cu. mm., with a normal differential and platelet count. The erythrocyte sedimentation rate (Wintrobe) was 3 mm. in one hour.

Radiography of the chest showed left ventricular enlargement. Plain films of the abdomen showed no abnormality other than the previously noted ankylosis of the sacroiliac joints. On intravenous pyelography, the excretory rate was normal on both sides. Symmetrically distributed circular opacities were to be seen in relation to all the calyces of both kidneys, which were of normal size. (Figs. 1, 2 and 3.) These opacities remained long after release of abdominal compression and represent cavities filled with contrast medium in the renal medulla. The diagnosis of medullary cystic disease was made on these appearances [*1,13*].

Renal clearances were performed according to the technics of Goldring and Chasis [*11*]. For chemical analyses the following methods were used: Inulin [*15*], para-amino-hippuric acid (PAH) [*16*], ammonium [*17*, titratable acid [*18*]. The urinary pH was measured on a Beckman pH meter. The concentrating ability of the kidney was assessed by the test of de Wardener [*19*].

The glomerular filtration rate (GFR), inulin clearance, was 124 ml. per minute (mean of five periods). Effective renal plasma flow (ERPF), para-amino-hippuric acid clearance, was 370 ml. per minute (mean of three periods). The maximal tubular excretory capacity for PAH was 44 mg. per minute (mean of two periods).

Table 1 compares the excretion of titratable acid and ammonium and the change in urinary pH after the oral administration of 6 to 7 gm. of ammonium chloride in the present case with the normal subjects of Wrong and Davies [*7*] and three normal subjects investigated in our laboratory. The results indicated a defective response in the excretion of ammonium. The specific gravity of serial specimens of urine after the subcutaneous injection of 5 units of pitressin tannate

FIG. 2. Enlarged view of left kidney.

FIG. 3. Line drawing of Figure 2.

TABLE 1

URINARY ACID EXCRETION

Load	Period	Hours										Mean Excretion in Last 8 Hours		
		1*	2*	3	4	5	6	7	8	9	10			
Ammonium (μEq./min.)	A	10.8	12.5	12.7	4.0	14.3	29.5	9.1	14.8	15.0	24.4	15.5		
	B	11.3	14.1	15.1	9.2	15.9	17.3	23.4	22.4	24.7	20.4	18.8	†	‡
	C		10.6									16.0	50.4 (33–75)	45.0 (38–52)
Titratable acid (μEq./min.)	A	11.2	10.1	8.0	20.6	26.7	43.0	26.5	31.4	19.4	31.4	26.6		
	B	18.7	18.7	19.9	31.7	55.6	41.4	40.7	30.1	39.1	48.0	38.3	†	‡
	C		12.7									29	37.9 (24–81)	40.0 (36–50)
pH	A	6.5	6.7	6.7	5.9	5.3	5.7	5.8	5.6	5.2	5.3	5.7		
	B	5.9	5.1	5.4	5.8	5.2	5.2	5.2	5.1	5.0	5.0	5.2		
	C	6.2										5.5		

NOTE: The acidifying function of the kidney was assessed by giving the patient 6 to 7 gm. ammonium chloride orally after a control period of two hours and measuring the urinary excretion of ammonium and titratable acid, and the urinary pH. The test was performed three times (A, B, C). On two occasions urine was collected hourly (by means of an indwelling catheter) for eight hours following the administration of the ammonium chloride. The plasma bicarbonate level at the midpoint of each test was 23 mEq. per L., 18.9 mEq. per L. and 20 mEq. per L., respectively.

* Control.

† Mean and range in ten normal subjects studied by Wrong and Davies [7].

‡ Mean and range in three normal subjects studied in this laboratory.

in oil was 1.010, 1.006, 1.012, 1.009, 1.010, 1.008, 1.010, 1.010 and 1.008. After a water load the urine was diluted to a specific gravity of 1.001.

COMMENTS

Clinical Features. Medullary cystic disease is more common in males and usually presents in early middle age [*1*], although cases have been reported in children [*20–22*]. Lindvall [*1*] states that the condition gives rise to no clinical manifestations except for those due to the complications of renal infection and calculi, the latter having been detected in thirty-three of his thirty-five cases. He makes no reference to the case of Smith and Graham [*20*] or to the four cases of Hogness and Burnett [*23*]. These had in common marked anaemia and insidious uraemia, usually without striking hypertension, cardiac enlargement or retinopathy. In three of them secondary hyperparathyroidism developed, but only one had calculi. These American reports stress the mild urinary abnormalities in the presence of severe uraemia. At necropsy all these patients had contracted kidneys with numerous cysts in the medulla. The widely divergent descriptions of these and Lindvall's cases suggest that different entities are being discussed but this may well be due to a study of the same condition at different stages in its evolution.

An interesting feature of the present case is the similarity in presentation on two admissions over five years apart. There had been very little progression in symptoms or signs except for the development of hypertension. The left ventricular enlargement cannot be ascribed to this alone as it was noted in 1954 in the absence of a raised blood pressure.

Radiological Features. Although no radiological illustrations were included in the report of Smith and Graham [*20*] they stated that an intravenous pyelogram demonstrated non-functioning kidneys and, judging by the description of the retrograde pyelogram, the radiological features of medullary cystic disease were not observed. Vermooten [*24*] described a patient showing the typical radiological features, the first report in the English language. The report of Hogness and Burnett [*23*] contained no reproductions of radiographs, but stated that one patient on retrograde pyelography showed "fuzzy" right middle and lower calyces with a similar appearance in the left lower calyx. Mulvaney and Collins [*2*] described a case of recurrent pyelonephritis in which medullary cysts were demonstrated in the left kidney by intravenous and retrograde pyelography. The condition was briefly discussed by Lagergren and Lindvall [*25*] in the differential diagnosis of renal papillary necrosis and was mentioned by Abeshouse and Salik [*26*] in a discussion of lesions of the renal papillae and calyces in cases of haematuria.

The first detailed description of the radiological features of medullary cystic disease was recorded by Lindvall in 1959 [*1*]. On intravenous pyelography the kidneys are normal in size or slightly enlarged and concentration of the opaque medium occurs normally unless there is gross renal damage. Cavities are seen in the renal pyramids and are round or cylindrical in shape. They may be bilateral and widespread or may affect only one calyx of a single kidney. According to Lindvall [*1*] the cysts are the first structures to fill with dye and they remain filled after release of ureteric compression but they may not be demonstrable on retrograde pyelography.

Renal Function. Little is known about renal function in the early stages of medullary cystic disease as patients studied so far were in a state of chronic renal failure. Smith and Graham [*20*] and Hogness and Burnett [*23*] have commented that uraemia develops insidiously and that urinary findings are sparse. In contradistinction to their cases the present patient showed no azotaemia and 3 plus proteinuria. In addition, numerous hyaline and granular casts were noted in the urine.

On detailed examination of kidney function in our case a normal GFR was found together with a decreased ERPF and Tm_{PAH}. This is characteristic of the renal haemodynamic state in both early essential hypertension [*11*] and chronic pyelonephritis [*27*]. However, the reaction of the kidney to an acid load was unusual and not characteristic of either of these conditions, and was of the type found in patients with chronic renal failure. Using the one day test as described by Wrong and Davies [*7*], a normal response was observed in that the urinary pH decreased and the titratable acid increased. On the other hand, the excretion of ammonium was well below the reported normal levels and below that of normal subjects investigated in our laboratory.

The test was performed on three occasions when the plasma bicarbonate level varied from 18.9 to 23 mEq. per L. Ammonium excretion roughly paralleled the systemic acidosis but never rose above the low level of 20 μEq. per

minute. Since the patient responded normally to the ammonium chloride load by a decrease in plasma bicarbonate and urinary pH and by an increase in urinary titratable acid, it must have been absorbed and constituted a sufficient stimulus. The fact that the basal ammonium excretion was not significantly different (Table 1) on a day when there was a mild systemic acidosis, as shown by a low basal plasma bicarbonate level (20.2 mEq. per L. in B of Table 1) compared with a day when the bicarbonate level was nearer the normal, is further evidence of impaired ammonium excretion.

There may be two reasons for the defective ammonium excretion. First it is possible that decreased renal blood flow resulted in an insufficient supply of substrate (glutamine and amino acids) for ammonia production, as has been suggested in cases of sodium depletion [*28*]. However, since the effective renal plasma flow was reduced by less than half and the maximal ammonium excretion of which the normal kidney is capable is at least ten times the figure found in this case [*29*] it would appear that decreased plasma flow was not the limiting factor. Moreover, patients with essential hypertension who have decreased renal plasma flow without diminished filtration rates have no defect in ammonia production.

The second possibility is that there is a localised defect at the site of the medullary cysts, i.e., in the collecting tubules. There is evidence that the latter are the site of ammonia production in mammals [*11,12*] but are not the site of hydrogen ion exchange. On intravenous pyelography it was thought that the lesion was symmetrically present in both kidneys. Possibly the hyperplastic cells [*30*] which line the dilated tubules do not possess the normal ability to produce ammonia due to an inherent functional defect. This favours the view that the collecting ducts are the site of ammonia production. An analogous situation, with cystic disease affecting another part of the kidney tubule, has recently been reported by Ward et al. [*31*] who found glycosuria without hyperglycaemia and lowered tubular reabsorption of inorganic phosphorus to be present in a patient with polycystic kidney thought to involve the proximal convoluted tubules. They did not measure the excretion of acid in their case.

It is possible that a disease unrelated to cyst formation such as chronic infection had affected the tubules. In support of this are the pathological findings in five previously reported cases [*20,23*] which are not incompatible with those seen in chronic pyelonephritis. Further, it is well known that polycystic disease of the kidney is liable to be complicated by infection [*32*]. There is, however, no good evidence of chronic pyelonephritis, repeated cultures of urine having been negative. Although this patient's original illness could well have been acute glomerular nephritis it would then appear that there was no relationship between the medullary cysts and this illness. As renal biopsy was not performed, because of its possible danger, the nature of the complicating condition, if any, cannot be identified.

There was an abnormal response to the injection of pitressin tannate in oil, renal concentrating ability being almost absent. This result may reflect an intrinsic inability to respond normally to circulating antidiuretic hormone and is compatible with a localised defect in the collecting tubules. The divergence of function seen here between concentration and dilution is also a feature of chronic pyelonephritis [*33*].

There are two other cases known to us in which low ammonia production was not associated with a marked depression of GFR [*34,35*]. In the earlier report a patient with Fanconi's syndrome is described who had a normal GFR but reduced ammonium excretion. The GFR was determined on the basis of one creatinine clearance only. The main feature of the second case, a patient with glomerulonephritis, was hyperkalaemia which was thought to be due to a specific defect in the potassium secretory mechanism. The excretion of ammonium did not increase when potassium excretion had returned to normal. In both these cases possible defects of function of the collecting tubules, apparently due to different etiologies, were associated with abnormalities of the proximal and/or the distal convoluted tubules.

Views as to the etiology of the condition do not appear to be helpful in establishing the reason for the tubular defect. The older opinions on the etiology of renal cysts, such as lack of embryonal fusion [*36,37*], tubular obstruction [*24,38*] and persistence of vestigial nephrons [*39*], have been discarded by Bailestok [*30*] as untenable. In an analysis of the morphogenesis of cystic disease she suggested that at a certain stage of embryonal life there is a disorder of development "due to a disturbance of organiser action and that this manifested by causing hyperplasia of the epithelium of the renal tubule causing cystic dilatation."

The finding of congenital abnormalities elsewhere in the body would support the opinion that there is a defect of development but there is only one such case report [*23*] in which medullary cystic disease was present in a twenty-eight year old man who had a club foot, congenital stenosis of the renal arteries and pancreatic cysts.

SUMMARY

The clinical, radiological and physiological features of a case of medullary cystic disease of the kidney are described.

The important observation made in this case was a defect in ammonium excretion in the presence of normal glomerular filtration. This is possibly indirect evidence that ammonia is produced in the collecting tubules.

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Hypogammaglobulinemia and Hypersplenism Associated with Lymphosarcoma of the Spleen*

Normal Serum Gamma Globulins Postsplenectomy

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HYPOGAMMAGLOBULINEMIA is a syndrome characterized by recurrent bacterial infections accompanied by a deficiency of plasma gamma globulin. There are three forms of this disease: (1) a congenital type usually seen in males; (2) an acquired type occurring in either sex and at any age; and (3) a transient form occurring in infancy as a result of inadequate gamma globulin synthesis in early life [*1*]. The acquired form of the disease may be idiopathic or secondary to neoplastic diseases of the reticuloendothelial system. In the idiopathic type, widespread reticulum cell hyperplasia associated with lymphadenopathy and hypersplenism has been found. The secondary variety of hypogammaglobulinemia has been seen with chronic lymphocytic leukemia, lymphosarcoma, thymoma, multiple myeloma and undifferentiated lymphomas. Therefore, in any patient with hypogammaglobulinemia and splenomegaly, with or without lymphadenopathy, the latter group of diseases must be considered [*2*].

The purpose of this report is to describe a patient with acquired hypogammaglobulinemia, secondary to lymphosarcomatous involvement of the spleen associated with hypersplenism whose serum gamma globulin levels returned to normal values five months after splenectomy. In addition, the patient has not had the complicating bacterial infections usually seen with this condition.

CASE REPORT

A. H. (Creighton University Dispensary No. ZB 95-02). A sixty-nine year old white male farmer had been followed up for many years at the Creighton University Dispensary for symptoms due to urinary retention secondary to prostatic hypertrophy. In May 1956 a non-tender splenic mass in the upper left quadrant of the abdomen was palpable 7 cm. below the costal margin. His hemogram was normal. In March 1958 he complained of a dragging sensation in the left side of the abdomen which was due to a huge splenic mass, extending 25 cm. below the left costal margin. His hematocrit ranged between 36 and 39 per cent, with a hemoglobin of 12 gm. per cent, a leukopenia of 3,600 to 5,000 white blood cells per cu. mm., and a platelet count varying between 160,000 to 225,000 per cu. mm. Bleeding and coagulation times, prothrombin time and acid phosphatase were all within normal limits. A serological test for syphilis had negative results. X-ray films of chest, pelvis, lumbar spine and skull were all within normal limits. Intravenous pyelogram revealed a medially displaced left kidney caused by the enlarged spleen. He was hospitalized and a transurethral resection performed because of persistent urinary symptoms.

Because the dragging sensation in his abdomen was worsening, the patient was hospitalized in April 1959. He emphatically denied having any bacterial infections during the past several years. His splenomegaly had increased to 27 cm. below the left costal margin and 2 cm. to the right of the midline. There was no lymphadenopathy or hepatomegaly. His course subsequent to this admission is summarized in Figure 1. Laboratory examinations revealed the hemoglobin to be 7.9 gm. per cent, hematocrit 30 per cent, white blood count 1,200 per cu. mm., reticulocytes 6 per cent. In the peripheral blood smear there was slight erythrocyte poikilocytosis, moderate anisocytosis and a considerable polychromasia—a pattern consistent with hemolytic anemia. Examination of the bone marrow showed markedly hyperplastic erythro-

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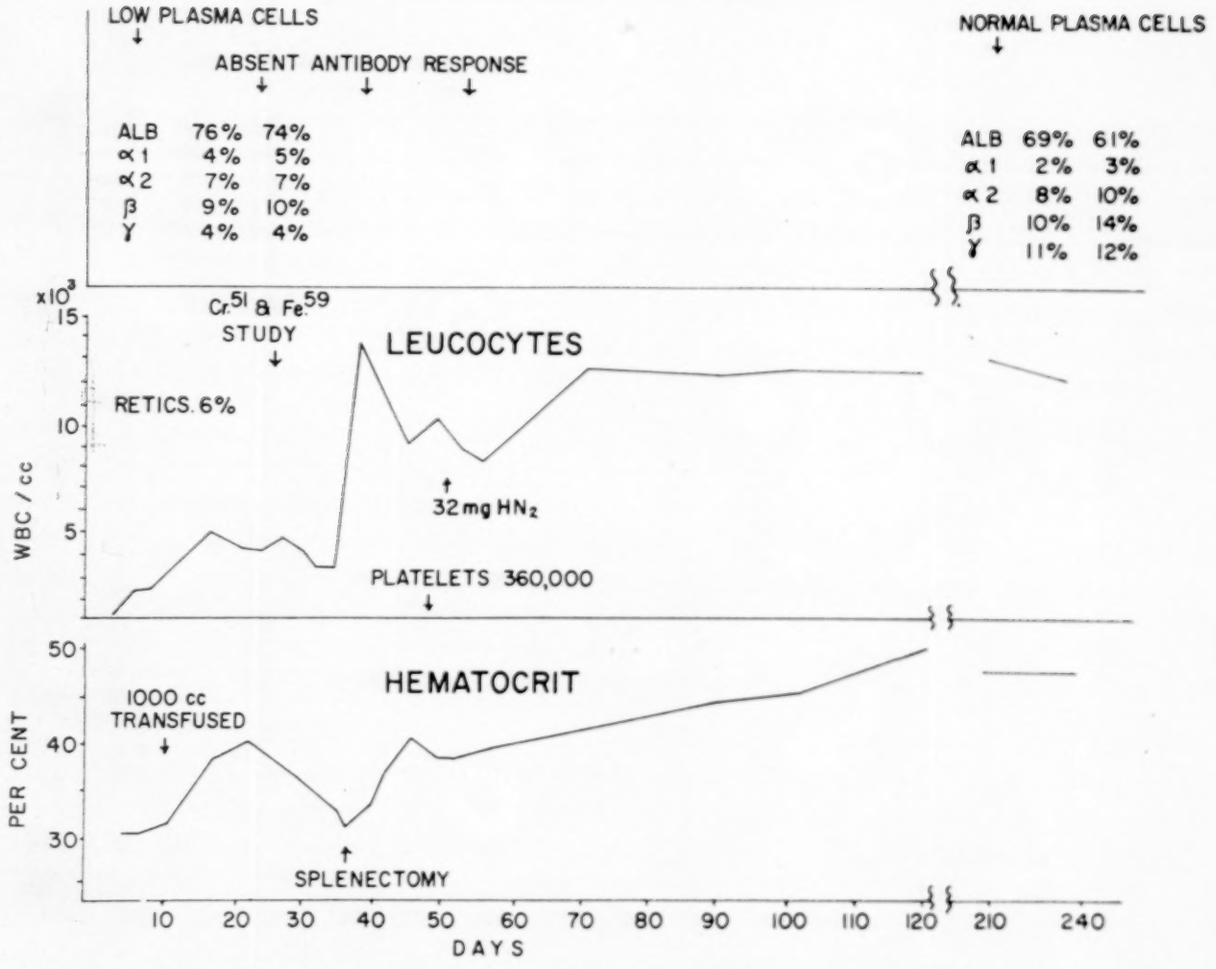

FIG. 1. Hospital course subsequent to April 1959. Data in the upper section correspond to time at the baseline.

poiesis with an erythroid-myeloid ratio of approximately 1:2, and increased iron stores. Leukopoiesis was normal. Plasma cells were strikingly diminished. Megakaryocytes were morphologically normal, but increased in numbers. Results of direct and indirect Coombs' tests were negative. A test for urine urobilinogen had negative results. Additional results were: serum albumin 5.4, globulin 0.8, total protein 6.2 gm. per cent; bromsulphalein 7 per cent retained in forty-five minutes; indirect bilirubin 1.2 mg. per cent, direct bilirubin zero, thymol turbidity test zero, cephalin flocculation test 1 plus in forty-eight hours, alkaline phosphatase 5.8 King-Armstrong units, serum glutamic pyruvic transaminase zero units, and serum glutamic oxaloacetic transaminase 17 units. Needle aspiration of the spleen revealed clumps of mature lymphocytes. Several mitotic figures were seen and the pattern was suggestive of malignant lymphoma. A Vim-Silverman needle biopsy of the liver showed periportal accumulations of lymphocytes but otherwise normal architecture. One month later a repeat biopsy of the liver revealed focal and scattered collections of mature lymphocytes throughout the hepatic parenchyma with no consistent lobular distribution. (Fig. 2.) This pattern was thought to be consistent with lymphocytic leukemia. A splenoportogram revealed dilatation of the splenic and portal veins (2.5 cm. in diameter) with dilated main divisions of the portal veins. The splenic pressures varied from 280 to 300 mm. Hg.

Filter paper electrophoresis, using the Durham type of apparatus, revealed a marked diminution of gamma globulin, as shown in Figure 3. Therefore, agglutination tests for typhoid O, typhoid H, paratyphoid A, paratyphoid B, Brucella abortus, heterophil antibody and cold agglutinins were made before and after challenge with 0.5 ml. of commercial typhoid-paratyphoid antigen; these revealed no agglutination. It was concluded that the patient was incapable of forming measurable antibodies. Results of tuberculin, histoplasmin and coccidioidin tests also were negative.

A simultaneous Cr^{51} and Fe^{59} isotope study was performed to evaluate erythropoiesis. Pulse height analysis was used to differentiate radioactivity due to each

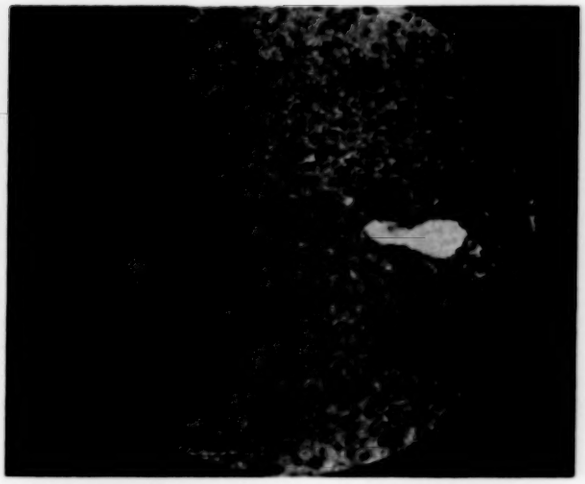

FIG. 2. Biopsy specimen of the liver showing focal and scattered collections of lymphocytes throughout the liver parenchyma.

isotope, similar to the method of Mitchell et al. [3]. *In vivo* studies with Fe^{59} over the liver, sternum, precordium and two splenic sites were performed. Iron turnover values were calculated, using the equations of Berlin et al. [4]. The results of the isotope study are presented in Table 1.

From the results given in Table 1, it is evident that the red cell volume was only slightly depressed. The mild anemia was hemolytic, as shown by a red blood cell half-life ($T^1/_2$) of seventeen days. The bone marrow was considered to be compensating for the hemolytic effect, since the red cell volume was maintained. Increased activity of the bone marrow is further substantiated by the rapid plasma iron disappearance, the greatly increased plasma iron turnover, the increased erythrocytes at four days, and the sharp, elevated rise over the marrow site on external counting for iron. While red blood cell survival was half normal,

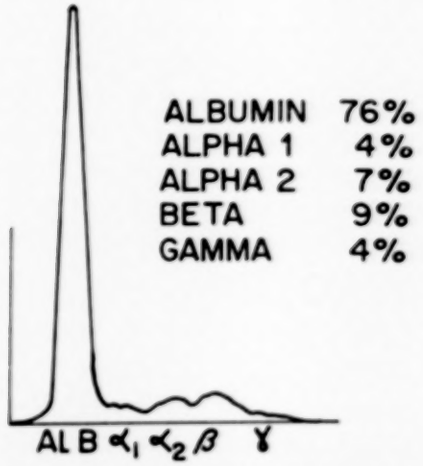

FIG. 3. Serum protein electrophoresis demonstrating a marked diminution in the gamma globulin fraction.

TABLE 1

RESULTS OF SIMULTANEOUS CHROMIUM^{51} AND IRON^{59} ISOTOPE STUDY

Subjects	Cr^{51} Blood Volumes			Cr^{51} Red Blood Cell Survival $T^1/_2$ (days)	Cr^{51} Organ Site Counting*		Postinjection Mixing of Cr^{51} Tagged Cells	Fe^{59} Plasma Disappearance $T^1/_2$ (minutes)	Fe^{59} Plasma Turnover		Fe^{59} Red Blood Cells Turnover		Fe^{59} Red Blood Cell Uptake
	Red Blood Cells (ml./kg.)	Plasma (ml./kg.)	Whole Blood (ml./kg.)		S:P Maximum	S:L Maximum			mg./day	mg./kg./day	mg./day	mg./kg./day	
Patient	26.3	53.4	80	17	1.56	1.99	Immediate mixing	34	124	1.4	150	1.69	100% at day 4
Normal subjects	29 ± 2	45 ± 5	75 ± 8	30 ± 6	<1.5	<1.0	Immediate mixing	70–90	21–37	0.3–0.4	14–13	0.22–0.28	70–90% at day 15

*S:P = spleen: precordium; S:L = spleen: liver.

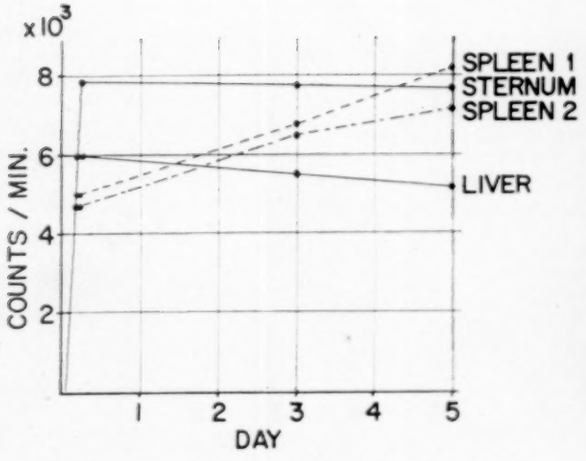

FIG. 4. Organ-site counting curves for iron[59].

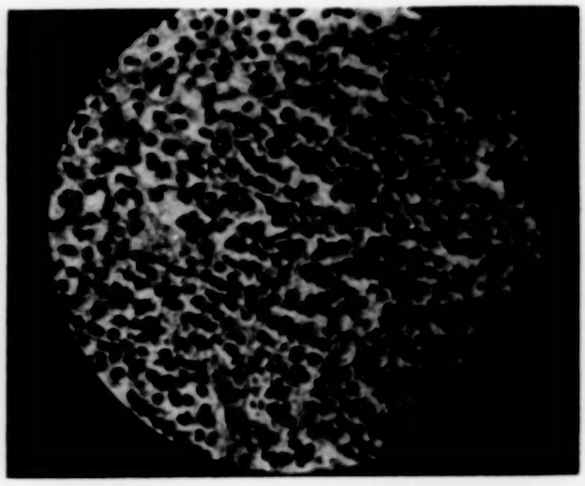

FIG. 5. Section of spleen showing lymphosarcoma.

the iron turnover was five to ten times greater than normal. There is no explanation for this quantitative discrepancy.

No evidence of delayed mixing of chromium-tagged erythrocytes indicative of a large pool of sequestered erythrocytes could be found in this patient [*5*]. The degree of sequestration seen grossly after splenectomy was large; however, an accurate estimate of intrasplenic blood volume was not made.

Evidence for splenic hemolysis was made apparent by the distribution of Cr^{51} activity on external counting. Increased splenic Cr^{51} activity is indicated by the high spleen:precordium (S:P) and spleen:liver (S:L) ratios. The values found in this patient are above the upper limits of normal for our series, and that of others [*6–8*]. (In twelve normal subjects, the S:L maximum was 1 and the S:P maximum was 1.4 in our series.)

The results of organ site counting for Fe^{59} are represented in Figure 4. Bone marrow uptake was rapid, and twice the liver uptake and 1.5 times the splenic uptake. There was no evidence of large hepatic uptake to suggest non-erythrogenic pooling of iron. The splenic uptake was normal in character between days one to three, with no evidence of splenic extramedullary hemopoiesis. The spleen incorporated large amounts of iron[59] after the third day, concomitant with marrow release of iron[59]-tagged erythrocytes. This pattern is consistent with splenic hemolysis, and has been called the "secondary splenic rise" by Emlinger and his group [*9*]. These studies indicated a splenic hemolytic anemia and marked improvement could be predicted after splenectomy.

On June 12, 1959, splenectomy was performed. No gross abnormalities were seen other than an extremely large spleen. The spleen weighed 5,700 gm. and was smooth. Microscopy revealed replacement of splenic architecture by uniform cells resembling mature lymphocytes morphologically. The normal follicular pattern was absent. A moderate number of mitotic figures were present. (Fig. 5.) The specimen was considered diagnostic of lymphosarcoma. A lymph node removed from the hilar region of the spleen was microscopically similar to the spleen, with penetration of cells beyond the capsule. A biopsy specimen of the liver obtained at surgery revealed accumulations of mature lymphocytes in the periportal areas with small clusters of lymphocytes widely scattered throughout the liver parenchyma, similar to that found on previous needle biopsy.

The postsurgical course has been uneventful. The hemogram has reverted to normal. (Fig. 1.) Repeat electrophoretograms five and six months after splenectomy have revealed a return of serum gamma globulin levels to normal and plasma cells have reappeared in the bone marrow. Enumeration of the number of plasma cells per 2,500 nucleated marrow cells showed two plasma cells in the preoperative as compared to twenty plasma cells in the postoperative specimens. The former value is within the range in patients with acquired lymphogammaglobulinemia reported by Good [*10*], while the latter value is completely normal.

COMMENTS

The patient described in this article had lymphosarcoma apparently isolated to the spleen, hypogammaglobulinemia demonstrated by paper electrophoretograms, and accompanying splenic hemolysis which was demonstrated by radioactive studies and confirmed by response to splenectomy. Postoperatively there was a complete return of serum gamma globulin levels and bone marrow plasma cells to normal.

While the patient had manifestations associated with hypogammaglobulemia, such as marked diminution of plasma cells in the bone marrow and lack of antibody production after

antigenic stimulus, he lacked the usual history of bacterial infections. The absence of bacterial infections, despite a marked deficiency of gamma globulin and lack of demonstrable antibody response to antigenic stimulus, conceivably could be explained on a quantitative basis. Perhaps the gamma globulin level in this patient was not depressed to levels at which susceptibility to infections occurs. The majority of patients with acquired hypogammaglobulinemia have gamma globulin concentrations between 25 and 75 mg. per 100 ml. [*1*]. The level in our patient was approximately 200 mg. per 100 ml. calculated on the percentage found in the electrophoretogram. In the literature a poor correlation between serum levels of gamma globulins and susceptibility to infections has been noted in individual cases [*11,12*].

The relationship of the malignant lymphomas and leukemias to hypogammaglobulinemia has been well documented. Wall [*2*] reports the incidence of hypogammaglobinemia (a reduction of gamma globulins of 30 per cent or more) to be 22 per cent in patients with malignant leukoses and only 1 per cent in all other diseases (excluding the nephrotic syndrome). As already indicated, in the majority of these cases the plasmocytic and lymphocytic cells are involved and such entities as chronic lymphocytic leukemia, lymphosarcoma, follicular lymphoma, thymoma, multiple myeloma and other related lymphomata are included, although rarely associated with Hodgkin's disease.

Most of the reported patients with hypogammaglobulinemia secondary to reticuloendothelial malignancies have had widespread involvement, suggesting replacement of the immunoprotein-synthesizing plasma and lymphocytic cells by leukemic or lymphomatous cells. On the other hand, several cases have been reported similar to the one described in this report, in which hypogammaglobulinemia has been associated with isolated regional tumor involvement [*13,14*]. The possibility that the lymphosarcoma itself or its effect on the spleen results in the release of an inhibitory factor to depress plasma cells, thereby resulting in lowered immunoprotein synthesis, must therefore be considered. As yet, no evidence for the occurrence of such mechanisms has been reported. Nonetheless, evidence has appeared for the preferential utilization of metabolic intermediates by rapidly metabolizing tumors, thereby depriving normal tissues [*15*]. In mice with spontaneous mammary carcinomas a specific depression of serum gamma globulin with subsequent beta globulin depression have developed [*16,17*]. The low serum globulin levels correlate with the size of the tumor.

SUMMARY

A patient with hypogammaglobulinemia and hypersplenism associated with (apparently) isolated lymphosarcoma of the spleen is presented. The patient was found to be incapable of forming measurable antibodies after antigenic challenge. Plasma cells in the marrow were diminished in number. Despite these findings, he had no history of bacterial infections.

Isotope studies demonstrated a splenic hemolytic process, which was substantiated by the satisfactory hematologic response postsplenectomy. The most remarkable finding was the complete return of serum gamma globulin levels and marrow plasma cells to normal after splenectomy.

Possible relationships between the tumors and alterations in immunoprotein synthesis are discussed.

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Pitressin-Resistant Diabetes Insipidus with Massive Hydronephrosis*

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THE polyuria and polydipsia of diabetes insipidus, hereditary or acquired, is usually due to lack of endogenous antidiuretic hormone (ADH) and therefore ordinarily is responsive to administration of exogenous pitressin. ADH is produced in the supraoptic and paraventricular nuclei of the hypothalamus by neurosecretory cells, is stored in the neurohypophysis [*4,5*] and released in response to increase in plasma osmolality [*43,44*] and to other stimuli, and probably acts by increasing the permeability of the renal distal and collecting tubules to water [*6,45*].

In some cases diabetes insipidus is refractory to treatment with the administration of Pitressin.® The underlying defect here is not lack of ADH, which in some instances has been identified in the urine or plasma [*12,22,28*], but is thought to be unresponsiveness of the renal tubule to Pitressin. The disorder in these cases is usually hereditary and the trait is transmitted as a sex-linked recessive, in contrast to transmission by simple dominance in the cases of hereditary diabetes insipidus in which there is a sensitivity to pitressin [*16,17*]. Pitressin-resistant diabetes insipidus usually appears in males [*7–30*] at an early age and may be associated with hydronephrosis, which is usually mild [*11,13,20*]. There are patients with syndromes which resemble Pitressin-resistant diabetes insipidus, but these are actually secondary to obstructive uropathy or other renal disease and may respond to correction of the underlying renal disorder [*31–37*].

This report is concerned with Pitressin-resistant diabetes insipidus in an adult, with onset in infancy. It was associated with massive hydronephrosis and a tendency to lose salt.

CASE REPORT

A thirty year old white farmer was admitted to the University of Minnesota Hospitals for the first time February 20, 1955 complaining of "kidney trouble." Polyuria, polydipsia and an enlarged bladder had first been noted at the age of six months, immediately after hospitalization for pneumonia, and have continued ever since. He was in a hospital for three weeks at the age of fourteen because of pneumonia, polyuria, polydipsia and urinary incontinence. The following year he was treated for dysuria and polyuria with antibiotics and intranasal Pitressin jelly for five weeks. Dysuria cleared but the polydipsia and polyuria continued unabated.

Five months prior to admission the patient was thought to have pyelonephritis, bilateral hydroureter and hydronephrosis, and bronchopneumonia. Four days prior to admission the patient noted bilateral flank pain, worse on the right side, dysuria, a feverish feeling and a moderate cough. There was no family history of polyuria or kidney disease. Two siblings died at early ages of unknown causes.

Physical examination revealed a thin dehydrated white man with deep respiration and occasional cough, who appeared acutely ill. The pulse was 112, respirations 30, temperature 103°F., blood pressure 115/80 mm. Hg. There was minimal clubbing and cyanosis of the fingers and moderate pharyngeal injection. Scattered rhonchi and wheezes were audible in the chest. There was tenderness in the deep right and left upper quadrant of the abdomen with no rebound, and bilateral costovertebral angle tenderness, more severe on the right. On rectal examination the prostate was of normal size.

Urine examination showed the following: specific gravity 1.003, pH 6, albumin 1 plus, sugar negative, 1 to 2 plus white cells, occasional red cells, and no casts. Hemoglobin was 19.6 gm. per cent, white blood cells 24,450 per cu. mm., with 89 per cent polymorphonuclear cells, 1 per cent lymphocytes and 10 per cent monocytes. The blood urea nitrogen was 57 mg. per cent; CO_2 20 mEq./L.; chloride 89 mEq./L.; sodium 123 mEq./L.; potassium 4.9 mEq. L.; total protein 6.8 gm. per cent; albumin, 2 gm. per cent; globulin, 4.8 gm. per cent; cholesterol, 128 mg. per cent with 60 mg. per cent esters. Blood culture was sterile. A pseudomonas species was isolated from the urine. Posteroanterior and lateral roentgenogram of

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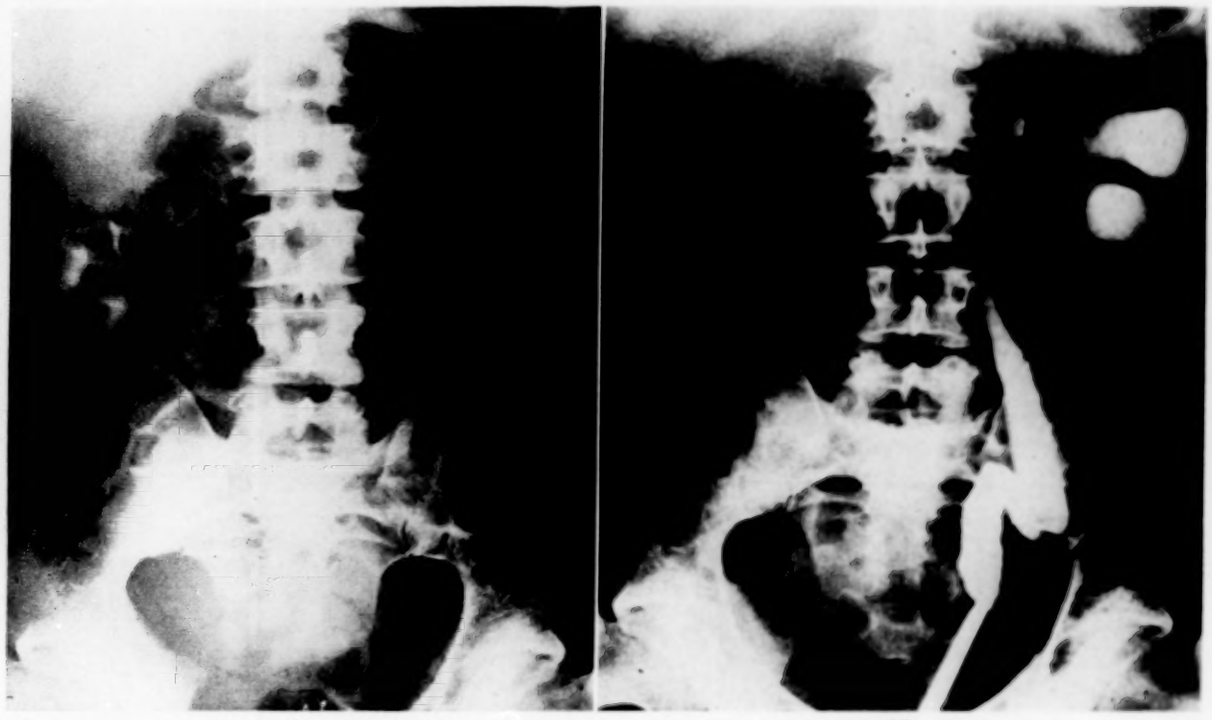

1A 1B

FIG. 1. Retrograde pyelograms of the right kidney (A), and left kidney (B), demonstrating bilateral hydronephrosis. Hydroureter is visualized for the left urinary tract. The upper urinary tracts are incompletely filled due to their large capacity.

the chest revealed an increase in density in the lower right lobe of the lung. The lungs became clear to auscultation, and costovertebral angle tenderness and fever disappeared after twenty days of therapy with procaine penicillin and Achromycin.® By the eighteenth day the white count had fallen to 10,200 per cu. mm. Repeated hemoglobin analyses ranged between 15.2 and 16.6 gm. per cent.

Roentgenograms of the skull revealed slight calcification of the pineal gland and habenular commissure, which were in the midline. Retrograde pyelograms revealed bilateral hydronephrosis and hydroureter. (Fig. 1.) At cystoscopy the urethra was normal up to the vesical neck where there was a collar-like hypertrophy. There was no prostatic enlargement. The trigone and ureteral orifices were normal. The bladder capacity was 1,000 cc. and there was mild trabeculation of the wall. Indigo carmine given intravenously was not seen to escape from either ureteral orifice after ten minutes. Urine from the right kidney was sterile; coagulase-negative staphylococci were isolated in broth from the urine of the left kidney.

Twenty-four hour urine volumes ranged from 4,400 to 26,000 cc. and were usually about 10,000 cc. Repeated urinary specific gravities ranged between 1.001 and 1.003. The patient was given a total of 124 pressor units of Pitressin intramuscularly over a three day period in divided doses, usually every four hours, from March 1 to March 4, with no change in urine volume or specific gravity from control levels. (Fig. 2.)

On March 8, 1955, following a nine hour period of water deprivation, a hypertonic saline infusion test was performed [*1*]. (Fig. 3.) The urine flow during the infusion of saline was greater than that during infusion of glucose and water, but the greatest output occurred during the period following cessation of the infusion. In normal subjects there is a drop in urine flow during saline infusion due to the stimulation of endogenous ADH secretion by the hypertonic saline solution [*1,2*].

The patient was discharged on March 11, 1955 and given 9 gm. of sodium chloride a day (because of low plasma sodium and chloride, presumably due to increased urinary loss). Body weight two days prior to discharge was 127 pounds.

On readmission March 21 to March 31, 1955, the sodium in the diet was restricted to 200 mg./day and water was given *ad libitum*. On this regimen the body weight fell from 143¾ to 136½ pounds, the subjective feeling of thirst decreased, and the output of urine varied from 6,800 to 12,000 cc. On admission the blood urea nitrogen was ten mg. per cent; CO_2 29 mEq./L.; chloride 99 mEq./L.; sodium 139 mEq./L.; potassium 4.2 mEq./L. Urine: specific gravity 1.001, trace albumin, no sugar or casts, and occasional white and red blood cells. The serum sodium fell to 133

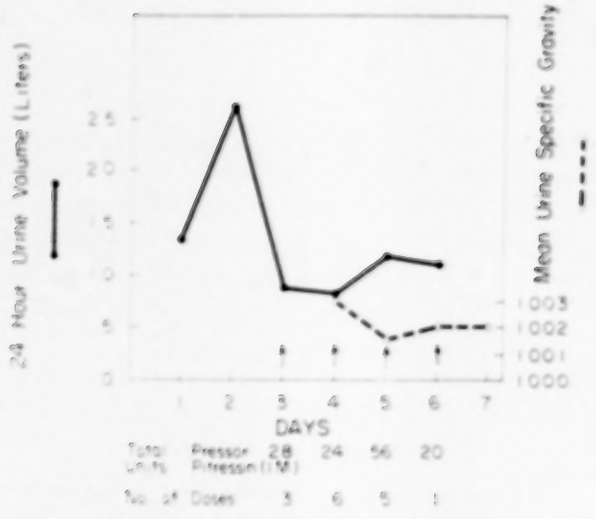

FIG. 2. Lack of effect of aqueous Pitressin therapy on urine volume and specific gravity.

(March 3, 1955) and 129 mEq. L. (March 28, 1955). The twenty-four hour urine sodium output on the ninth day of admission was 600 mg.

During twenty-two hours of complete water and food deprivation the output of urine was 5,071 cc., body weight dropped from 136½ pounds to 126¾ pounds, and urine specific gravities ranged from 1.001 to 1.002. (Table 1, Test 1.) Serum sodium after 4,000 cc. had been excreted during the period of water deprivation was 144 mEq./L. A test infusion of hypertonic saline solution was again given following water and food deprivation and yielded results similar to those obtained previously. (Fig. 3.) Urine specific gravities were 1.002 throughout the infusion.

After ingestion of 45 gm. of sodium chloride (15 gm. daily) the patient was again deprived of all oral intake for twenty-two hours during which weight dropped from 149 to 125¾ pounds, urine output was 9,355 cc., while the specific gravities of urine ranged from 1.001 to 1.002. (Table 1, Test 2.) A serum sodium taken after at least 6,000 cc. of output was 169 mEq./L. There was marked thirst during all periods of water deprivation, accompanied by loss of substance from the face, extreme weakness and sometimes a feeling of faintness on arising.

The patient has been seen every six months through 1960 and has continued to drink two to three gallons of water per day, voiding about every one and a half hours during the day and every two hours at night. The plasma chloride concentration rose to 126 mEq./L. on a regimen of 9 gm. of supplementary sodium chloride per day, and fell to normal levels when the supplement was changed to sodium chloride, 6 gm., and sodium bicarbonate, 2.6 gm., daily. The residual urine varied from 200 to 1,000 cc. A cystogram on June 17, 1959, revealed an enlarged bladder with saccules and trabeculations. The urethrogram showed

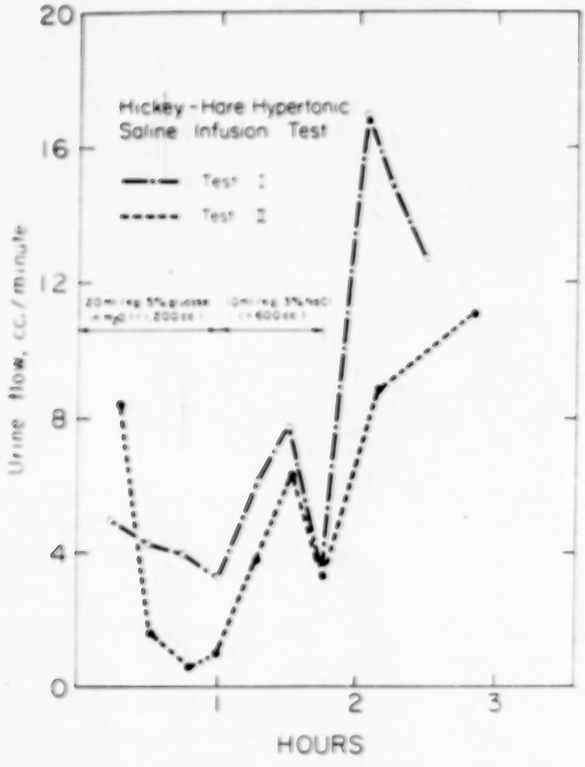

FIG. 3. Effect of the administration of 3 per cent sodium chloride on water diuresis. The antidiuresis seen in normal subjects did not occur.

TABLE 1
EFFECT OF WATER DEPRIVATION ON URINE VOLUME AND SPECIFIC GRAVITY AND BODY WEIGHT

Time Period (hr.)	Mean Urine Flow (cc./hr.)	Urine Specific Gravity	Body Weight (lbs.)	Comments
Test 1 (March 31, 1955)				
Twelve hour period just prior to beginning of test	650	...	136.5	Water *ad libitum*
0–12*	230	1.001	127.5	Nothing by mouth
12–14	550	1.001	...	Nothing by mouth
14–16	210	1.001	...	Nothing by mouth
16–18	120	1.002	...	Nothing by mouth
18–20	188	1.001	...	Nothing by mouth
20–21.75	100	1.002	126.75	Nothing by mouth
Test 2 (April 3, 1955)				
0–10*	465	1.001	149	Nothing by mouth
10–12	665	1.002	...	Nothing by mouth
12–14	520	1.002	...	Nothing by mouth
14–16	350	1.002	...	Nothing by mouth
16–18	198	1.002	128.5	Nothing by mouth
18–20	325	1.002	...	Nothing by mouth
20–22	295	1.001	125.75	Nothing by mouth

* Beginning of test.

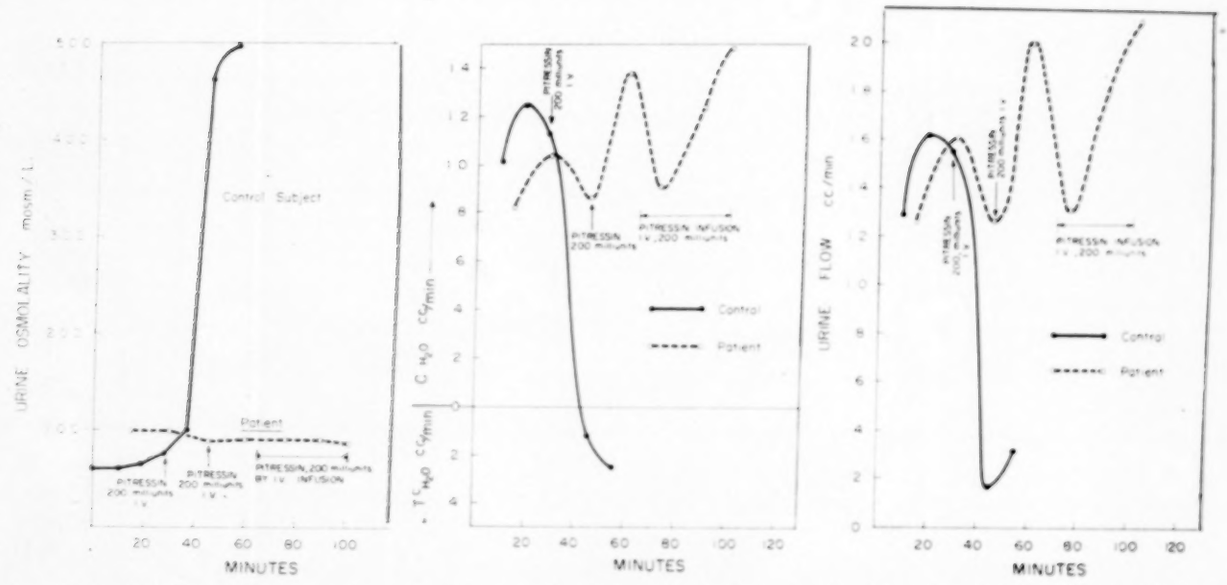

FIG. 4. Effect of Pitressin therapy on water diuresis and urine osmolality in patient and in control subject. The ordinates in the middle panel are C_{H_2O} (free water clearance) and $T^C_{H_2O}$ (free water absorption).

no urethral obstruction. The patient has been feeling quite well on this program.

A trial of Pitressin snuff given three times daily for one month was without effect on the polyuria and polydipsia. During a water diuresis the administration of intravenous Pitressin was ineffective in increasing urine osmolality, in decreasing the volume of urine and in eliminating free water clearance in our patient; it produced the anticipated antidiuretic effect in a control subject. (Fig. 4.) Urea clearances ranged from 45 to 60 cc./min. (mean 51, mean normal value, 75 cc./min.). The creatinine clearance was 109 cc./min.

COMMENTS

This patient appears to demonstrate an authentic example of Pitressin-resistant diabetes insipidus with obligatory polyuria and polydipsia. First, no endogenous antidiuretic response could be elicited by hypertonic saline solution (Hickey-Hare test) or by twenty-two and twenty-four hours of water deprivation, which resulted in a marked loss of body weight and profound thirst. Secondly, the administration of Pitressin failed to alter urinary flow, specific gravity or osmolality. Presumably the patient has endogenous ADH to which the kidney is not responsive.

The onset at the age of six months probably puts this case in the category of Pitressin-resistant diabetes insipidus found in male infants and transmitted as a sex-linked recessive by females [*11*]. Although there is no familial history of similar disease, the unexplained death in infancy of two male siblings suggests this possibility. The usual clinical picture in children consists of onset shortly after birth, unexplained fevers, constipation, vomiting, polydipsia and polyuria unresponsive to Pitressin, elevated serum sodium and chloride (with insufficient hydration), high skin resistance, rapid dehydration on withholding fluids, excretion of a urine of low specific gravity, together with a familial incidence and occurrence usually in males [*10*]. Clinically unaffected female ancestors are not able to concentrate urine to as high a level as normal persons in response to Pitressin [*25,28*]. Normal [*10,28*] and abnormal [*11,22*] studies of renal function have been reported in Pitressin-resistant diabetes insipidus.

The finding of Pitressin-resistant diabetes insipidus in an adult associated with massive hydronephrosis at first suggested the diagnosis of water-losing nephritis due to hydronephrosis [*32*], but the subsequent clarification of the onset at age six months, the extremely dilute urine, and the lack of sufficient anatomic obstruction to have caused hydronephrosis as a primary event makes this cause unlikely. The "water-losing" in some of these cases has been reversed by eliminating the hydronephrosis [*32,35,37*]. In one case the urine was twice as dilute from one kidney as the other. It has been suggested that

these Pitressin-resistant polyurias are due to renal tubular damage resulting from increased intratubular pressure [*32*]. There is a report associating the presence of lesions in the tuber cinereum with the onset in adults of a Pitressin-resistant polyuria of low specific gravity [*38*]. Inasmuch as polyurias and thirst mechanisms are not fully understood, this mechanism would not appear to be ruled out in our case. However, direct action of Pitressin on the isolated kidney, free from neural connection, and perfused through a heart lung preparation has been demonstrated [*46*]. Renal unresponsiveness to Pitressin would therefore appear to be the most likely mechanism in Pitressin-resistant diabetes insipidus.

Bladder capacity of over 1,000 cc. (without discomfort) and enormous hydronephrosis and hydroureter have been reported in a number of cases of hereditary diabetes insipidus (without mention of Pitressin-resistance) [*13*] and in other cases similar to our patient, which were resistant to posterior pituitary extract [*39*]. The hydronephrosis probably results from the large urine flows and probably the failure of the bladder to enlarge sufficiently to contain at normal pressures the volume of urine excreted between periods of voiding. Overdistention of the bladder leading to hypotonicity of the bladder musculature and residual urine aggravates the situation. In this regard it is interesting that in a strain of mice with primary polydipsia the combination of an abnormally high output of urine in association with the partial urethral obstruction which is normally present in all male mice has been shown to be an essential factor in the production of the hydronephrosis encountered in a high percentage of these mice [*40*].

Our patient exhibited abnormal handling of salt. On the one hand, plasma sodium and chloride were elevated in the presence of high salt intake. On the other hand, with a normal intake of salt there was a negative salt balance and decreased plasma sodium and chloride concentrations, even in the presence of dehydration. The patient also demonstrated an inability to excrete sufficient chloride in excess of sodium to prevent hyperchloremia when given large amounts of supplementary sodium chloride. Fever and hyperelectrolytemia, but not salt-losing, is a common feature in childhood cases of Pitressin-resistant diabetes insipidus, and may be contributed to by a lack of thirst in the first year of life [*26*]. This has been treated by decreasing solute intake and increasing fluid intake [*27*]. The onset of the polyuria at six months of age in the case presented herein may simply coincide with the onset of thirst. The pattern of poor salt handling probably results at least in part from the kidney damage due to hydronephrosis. It is similar to that described in patients with advanced glomerulonephritis, arteriosclerotic renal disease and chronic suppurative nephritis [*41*], but is not as severe as that described as salt-losing nephritis [*42*].

SUMMARY

Pitressin-resistant diabetes insipidus, with massive bladder enlargement and bilateral hydroureter and hydronephrosis, in an adult is described. The pertinent literature is reviewed.

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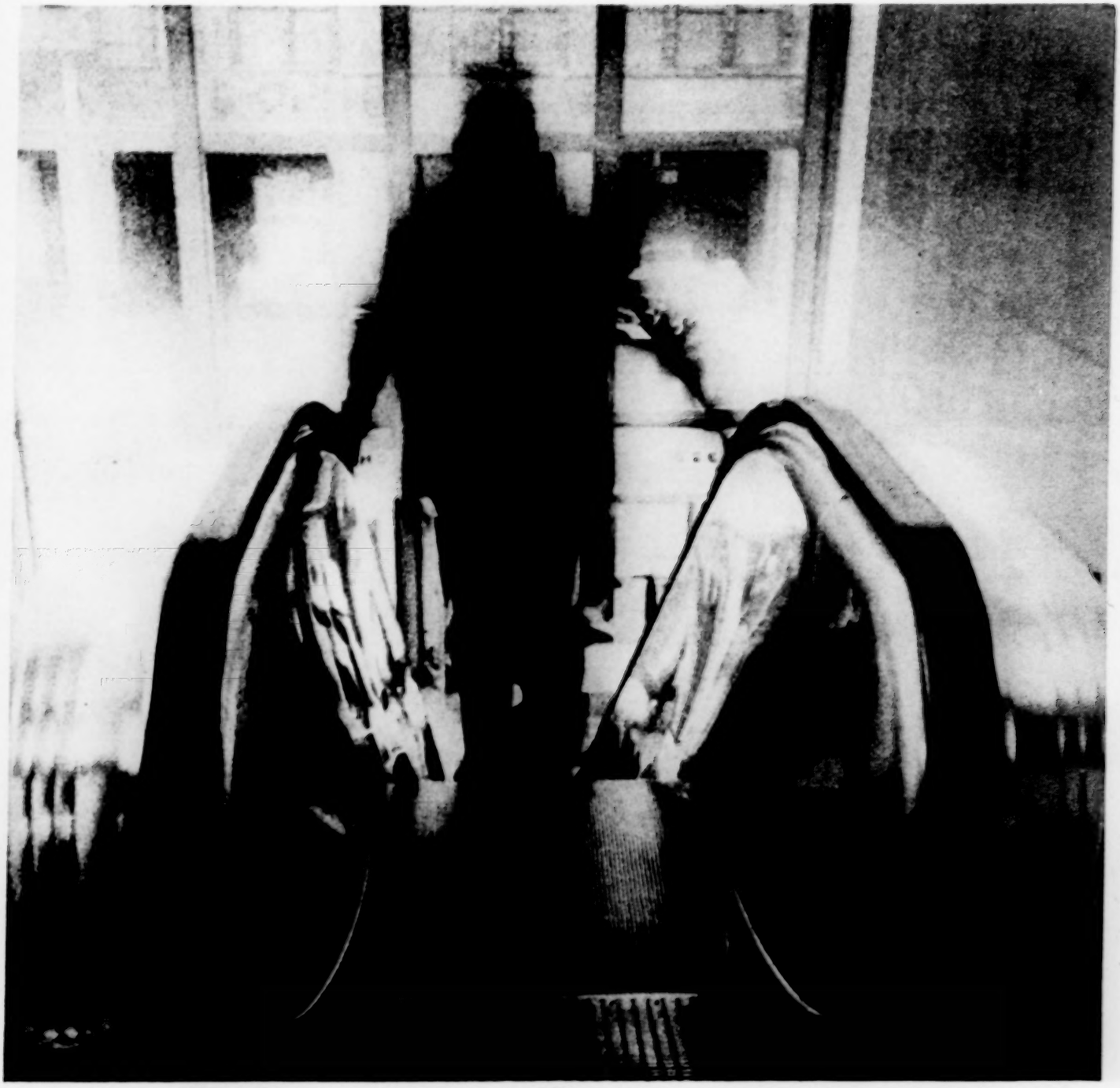

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in any rheumatic 'itis'

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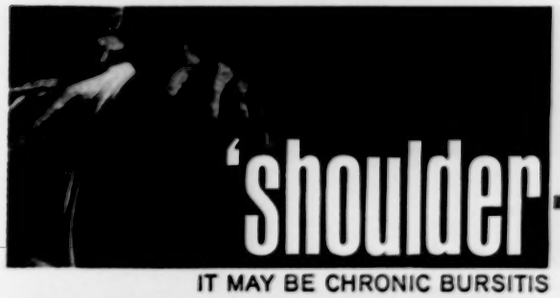

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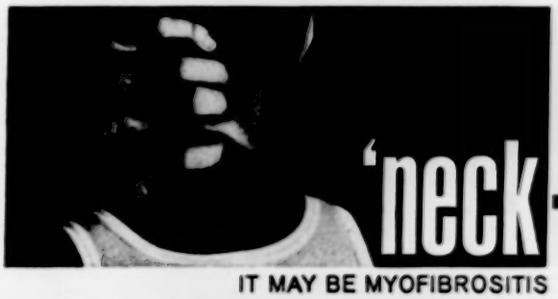

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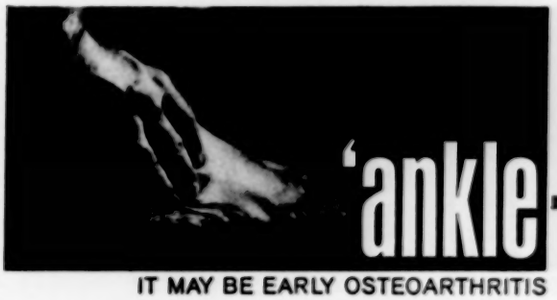

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METICORTEN® (prednisone)	safer, reduced dosage	0.75 mg.
Acetylsalicylic acid	supportive anti-inflammatory-analgesic	325 mg.
Aluminum hydroxide	a buffer for better toleration	75 mg.
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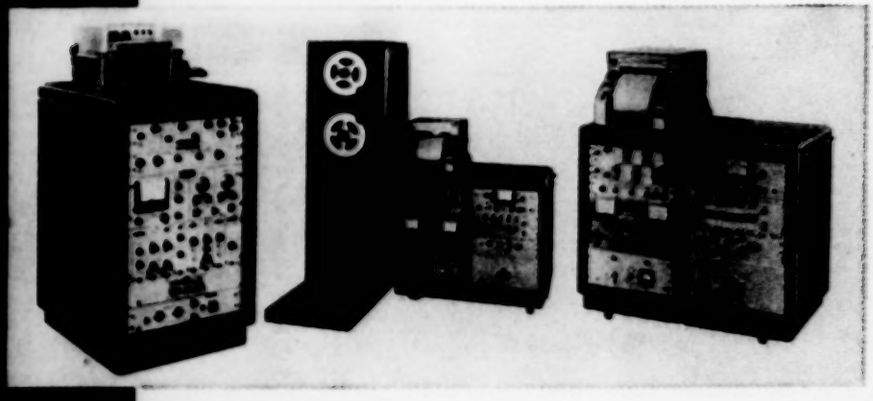

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no "cumulative sedative drag" Since these ingredients have essentially the same duration of action, this makes possible an even, time-matched therapy for balanced control of both tension and spasm, without the "cumulative sedative drag" so many patients experience with phenobarbital.

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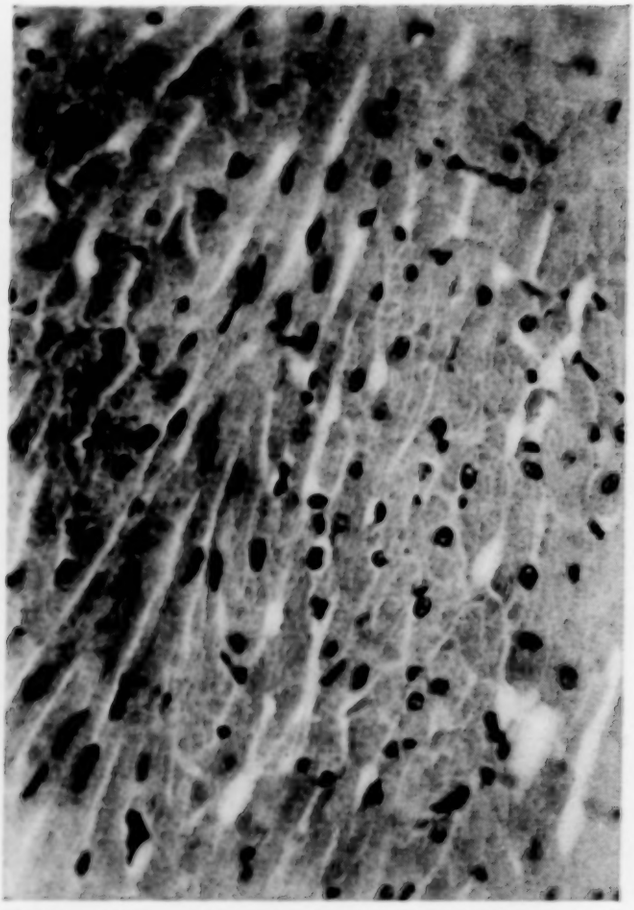
New laboratory evidence shows Serpasil® prevents heart damage

Severe heart damage in unprotected stressed rat. Tissue taken from rat given 2-α-methyl-9-α-fluorohydrocortisone and stressed by restraint. Photomicrographs from Raab et al.* (Original magnification: approximately 450X.)



No heart damage in stressed rat protected with Serpasil. Tissue taken from rat given 2-α-methyl-9-α-fluorohydrocortisone and stressed as at left, but also given Serpasil (0.4 microgram daily for one week).

Note: While Serpasil did not completely protect the hearts of all animals in this study, it greatly reduced myocardial damage in most of them.

This evidence suggests that Serpasil may protect your hypertensive patient's heart.

Complete information about indications, dosage, precautions and side effects will be sent on request.

Supplied: *Tablets,* 0.1 mg., 0.25 mg. (scored).

*Raab, W., Stark, E., and Gigee, W. R.: Unpublished data.

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Can a plumber do a day's work on 1200 calories?

The answer, of course, is "not for long." For example, following diagnosis of diabetes, a 44-year-old plumber (5'8" and weighing 147 lb.) had been put on a 1200-calorie diet to control glycosuria. When referred six months later, he had not been spilling sugar, but had lost 25 pounds and developed progressive fatigability. Orinase, 0.5 Gm./day, was prescribed and his diet was increased to 2800 calories to meet metabolic demands (125 Gm. protein; 300 Gm. carbohydrate; 125 Gm. fat).

Follow-up visits showed this progress:

3 mo. Urine and blood sugar o.k.; weight gain: 28 lb. Can work normally, feels generally well.

6 mo. Weight constant, control constant, no complaints.

12 mo. Same.

18 mo. Same.

24 mo. Same.

Diet-controlled diabetics who are underweight, tire easily, or have increased nutritional needs may merely be "getting by" on dietotherapy alone. These patients – and others who experience transient weakness or listlessness–can often be returned to near-normal activity by giving Orinase together with a more adequate diet. Orinase control of diabetes is notably smooth and stable; patients report a greater sense of well-being, an improved mood and outlook.

Case data courtesy Henry Dolger, M.D.

Indications and effects: The clinical indication for Orinase is stable diabetes mellitus. Its use brings about the lowering of blood sugar; glycosuria diminishes, and such symptoms as pruritus, polyuria, and polyphagia disappear.

Dosage: There is no fixed regimen for initiating Orinase therapy. A simple and effective method is as follows: First day – 6 tablets; second day – 4 tablets; third day – 2 tablets. The daily dose is then adjusted – raised, lowered or maintained at the two-tablet level, whichever is necessary to maintain optimum control.

In patients being converted from insulin, insulin is gradually withdrawn in accordance with the response to Orinase observed over a trial period that may extend to three or four weeks. In candidates for combined Orinase-insulin therapy, an individualized schedule is usually obtainable during a trial course of two or more weeks.

Contraindications and side effects: Orinase is contraindicated in patients having juvenile or growth-onset, unstable or brittle types of diabetes mellitus; history of diabetic coma, fever, severe trauma or gangrene.

Side effects are mild, transient and limited to approximately 3% of patients. Hypoglycemia and toxic reactions are extremely rare. Hypoglycemia is most likely to occur during the period of transition from insulin to Orinase. Other untoward reactions to Orinase are usually not of a serious nature and consist principally of gastrointestinal disturbances, headache, and variable allergic skin manifestations. The gastrointestinal disturbances (nausea, epigastric fullness, heartburn) and headache appear to be related to the size of the dose, and they frequently disappear when dosage is reduced to maintenance levels or the total daily dose is administered in divided portions after meals. The allergic skin manifestations (pruritus, erythema, and urticarial, morbilliform, or maculopapular eruptions) are transient reactions, which frequently disappear with continued drug administration. However, if the skin reactions persist, Orinase should be discontinued.

Clinical toxicity: Orinase appears to be remarkably free from gross clinical toxicity on the basis of experience accumulated during more than four years of clinical use. Crystalluria or other untoward effects on renal function have not been observed. Long-term studies of hepatic function in humans and experience in over 600,000 diabetics have shown Orinase to be remarkably free of hepatic toxicity. There has been reported only one case of cholestatic jaundice related to Orinase administration, which occurred in a patient with pre-existing liver disease and which rapidly reversed upon discontinuance of the drug.

Each tablet contains:
Tolbutamide ..... 0.5 Gm.
Supplied: In bottles of 50.

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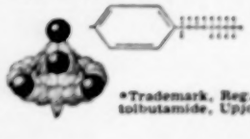
An exclusive methyl "governor" prevents hypoglycemia.



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The suspension contains pyrvinium pamoate equivalent to 10 mg. pyrvinium base per cc. The sugar-coated tablets contain pyrvinium pamoate equivalent to 50 mg. pyrvinium base. *Dosage:* Children and adults, a single oral dose equivalent to 5 mg. per Kg. Not appreciably absorbed from the gastrointestinal tract. *Precautions:* Infrequent nausea and vomiting and intestinal complaints have been reported. Tablets should be swallowed whole to avoid staining teeth. Will color stools a bright red.

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NOTE: Each 'Cardioquin' Tablet contains the quinidine-equivalent of the conventional three-grain tablet of quinidine sulfate, thus providing facility in dosage calculation or substitution for other quinidine salts. 'Cardioquin' Tablets may be substituted one tablet for each three-grain quinidine sulfate tablet (or equivalent) previously administered.

Consult Product Data Brochure, available on request, for complete directions, contraindications and precautions attending the use of the drug.

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Complete information on RELA including indications, dosage, side effects, and precautions is available to physicians on request.

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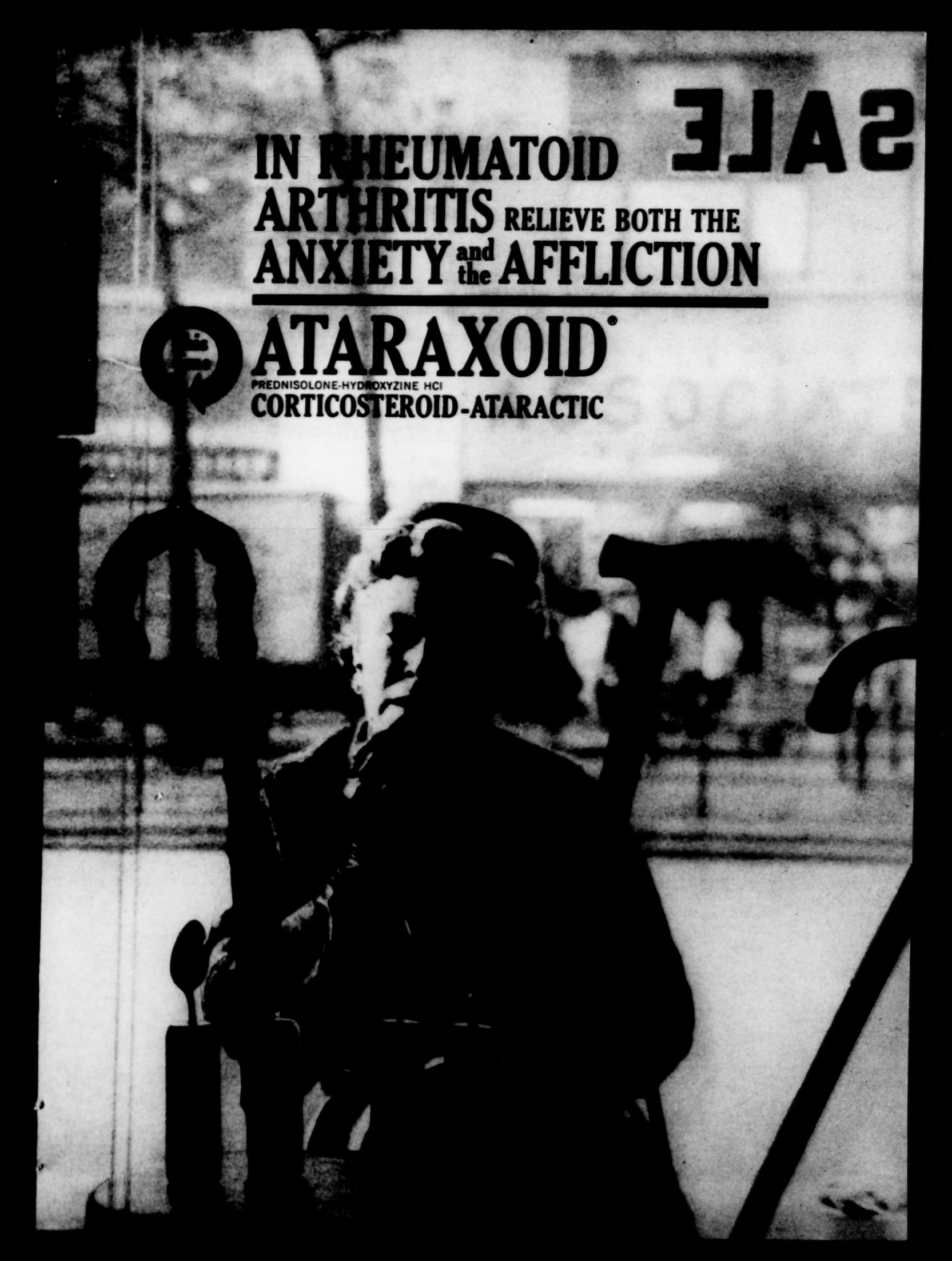
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Erythropoietin—a significant discovery in clinical hematology

A wealth of evidence now confirms the fact that red blood cell production is controlled by the hormone erythropoietin.[1-3] Demonstrated in human plasma,[4] erythropoietin has been shown to produce reticulocytosis,[1,5-7] increase utilization of the Fe^{59} isotope, and increase erythrocyte precursors in marrow cultures.[3,8]

ERYTHROPOIETIN FOUND TO CONTROL RED CELL FORMATION

erythropoietin levels—new criteria in diagnosis of anemia—Increased erythropoietin blood levels can be demonstrated in severe anemia and following the start of accelerated formation.[9] Soon thereafter, the effect of the higher levels appears as an increased erythroid marrow activity.[10] Since the hemopoietic marrow is capable of producing more red cells than normally required, many anemias may be due to inadequate erythropoietin levels—a result of subnormal production or excessive excretion.

how does erythropoietin affect iron metabolism? Absorption and utilization of iron are dependent upon the rate of bone marrow erythropoiesis which, in turn, is dependent upon erythropoietin levels.[11,12] Thus, the demand for iron created by accelerated erythropoiesis is satisfied by both increased gastrointestinal absorption and mobilization of storage iron. Inadequate erythropoietin levels would seemingly account for the frequently disappointing results with the use of iron alone in many of the anemias.

can medication increase erythropoietin levels? Cobalt has been shown to be strikingly effective in increasing the production of erythropoietin.[13,14] Cobalt-enhanced erythropoietin accelerates red cell production and improves iron utilization with a subsequent increase in hemoglobin and erythrocytes. The new concepts of the cause, diagnosis, and management of anemia may now be applied clinically on the sound basis of extensive studies published on RONCOVITE®—MF, the therapeutic cobalt-iron hematinic.

(1) Gordon, A. S.: Physiol. Rev. **39**:1, 1959. (2) Erslev, A. J.: J. Lab. & Clin. Med. **50**:543, 1957. (3) Rosse, W. F., and Gurney, C. W.: J. Lab. & Clin. Med. **53**:446, 1959. (4) Gurney, C. W.; Goldwasser, E., and Pan, C.: J. Lab. & Clin. Med. **50**:534, 1957. (5) Rambach, W. A.; Alt, H. F., and Cooper, J. A. D.: Blood **12**:1101, 1957. (6) Gordon, A. S., **et al.**: Proc. Soc. Exp. Biol. & Med. **92**:598, 1956. (7) Erslev, A. J.: Blood **10**:954, 1955. (8) Goldwasser, E.; Jacobson, L. O.; Fried, W., and Plzak, L. F.: Blood **13**:55, 1958. (9) Stohlman, F., Jr., and Brecher, G.: Proc. Soc. Exp. Biol. & Med. **100**:40, 1959. (10) Kraus, L. M., and Kraus, A. P.: Fed. Proc. **18**:1051, 1959. (11) Bothwell, T. H.; Pirzio-Biroli, G., and Finch, C. A.: J. Lab. & Clin. Med. **51**:24, 1958. (12) Beutler, E., and Buttenwieser, E.: J. Lab. & Clin. Med. **55**:274, 1960. (13) Goldwasser, E.; Jacobson, L. O.; Fried, W., and Plzak, L.: Science **125**:1085, 1957. (14) Murdock, H. R., Jr., and Klotz, L. J.: J. Am. Pharm. A. (Scient. Ed.) **48**:143, 1959.

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Supplied: Each **Ambar #2 Extentab** contains: Methamphetamine HCl 15 mg., phenobarbital 64.8 mg. (1 gr.). Each **Ambar #1 Extentab** contains: Methamphetamine HCl 10.0 mg., phenobarbital 64.8 mg. (1 gr.). One Extentab® before breakfast provides appetite and mood control for 10 to 12 hours, in a single, controlled-release, extended action tablet.

Also available are regular **AMBAR TABLETS.** Each contains methamphetamine HCl 3.33 mg., phenobarbital 21.6 mg., (⅓ gr.). For use in conventional dosage schedules (one or two t.i.d.), or for intermittent or supplemental therapy.

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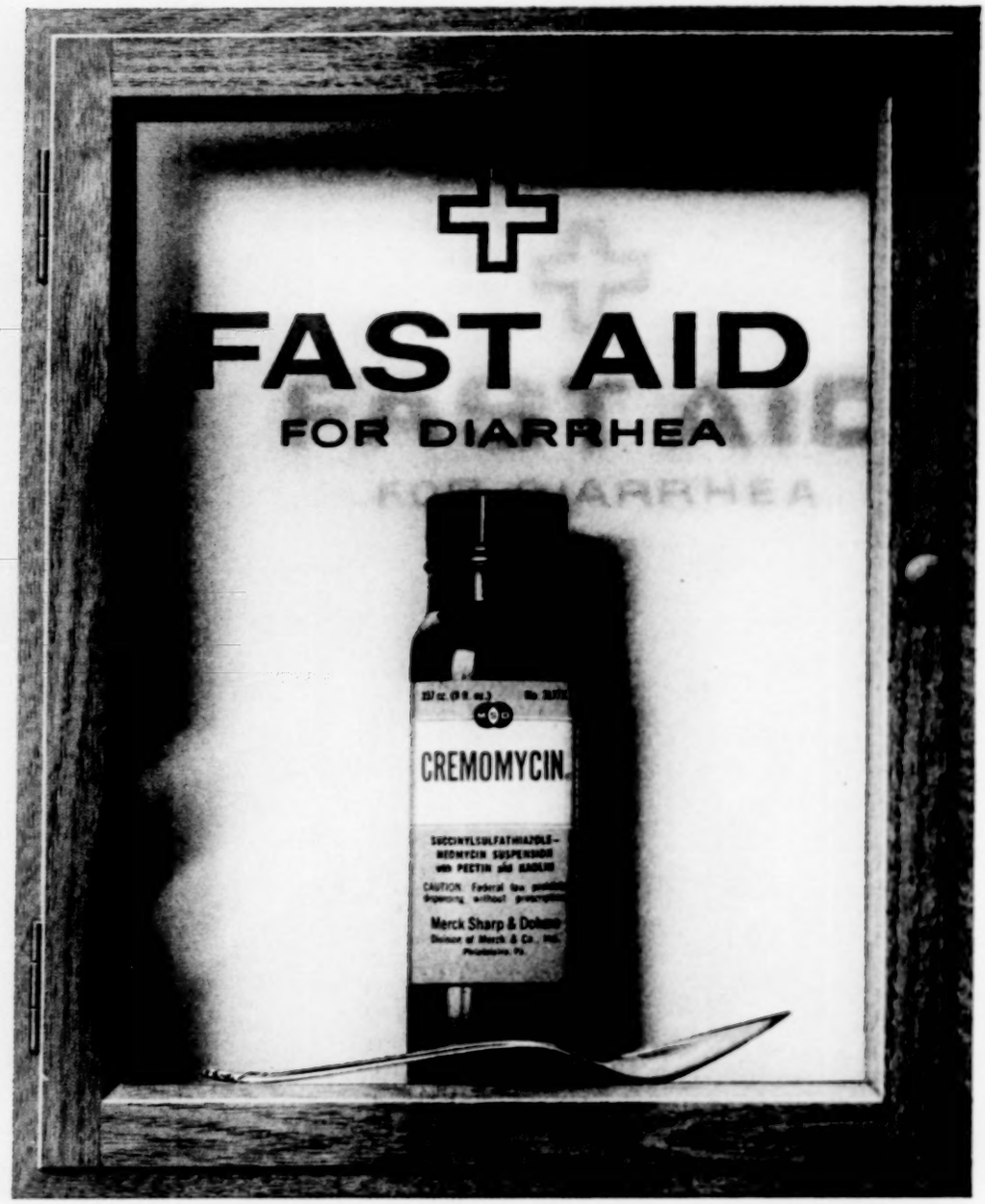

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UNSURPASSED "GENERAL-PURPOSE" CORTICOSTEROID . . .

Aristocort®

Triamcinolone LEDERLE

OUTSTANDING FOR "SPECIAL-PURPOSE" THERAPY

Lederle

Aristocort

Triamcinolone has long since proved its unsurpassed efficacy and relative safety in the therapy of *rheumatoid arthritis, inflammatory and allergic dermatoses, bronchial asthma,* and all other conditions in which corticosteroids are indicated. But ARISTOCORT has also opened up new areas of therapy for selected patients who otherwise could not be given corticosteroids. Medicine is now in an era of "special-purpose" steroids.[1]

One outstanding advantage of triamcinolone is that it rarely produces edema and sodium retention.[1, 2]

The clinical importance of this property cannot be overemphasized in treating certain types of patients. McGavack and associates[3] have reported the beneficial results with ARISTOCORT in patients with existing or impending cardiac failure, and those with obesity associated with lymphedema. Triamcinolone, in contrast to most other steroids, is not contraindicated in the presence of edema or impending cardiac decompensation.[3]

Hollander[1] points out the superiority of triamcinolone in not causing mental stimulation, increased appetite and weight gain, compared to other steroids which produce these effects in varying degrees. And McGavack,[2] in a comparative tabulation of steroid side effects, indicates that triamcinolone does not produce the increased appetite, insomnia, and psychic disturbances associated with other newer steroids.

ARISTOCORT can thus be advantageous for patients requiring corticosteroids whose appetites should not be stimulated, and for those who are already overweight or should not gain weight. Likewise, ARISTOCORT is suitable for the many patients with emotional and nervous disorders who should not be subjected to psychic stimulation. Furthermore, ARISTOCORT Triamcinolone, in effective doses, showed a low incidence of side reactions and is a steroid of choice for treating the older patient in whom salt and water retention may cause serious damage.[2]

References: 1. Hollander, J. L.: *J.A.M.A.* 172:306 (Jan. 23) 1960. 2. McGavack, T. H.: *Nebraska M. J.* 44:377 (Aug.) 1959. 3. McGavack, T. H.; Kao, K. Y. T.; Leake, D. A.; Bauer, H. G., and Berger, H. E.: *Am. J. M. Sc.* 236:720 (Dec.) 1958.

Precautions: Collateral hormonal effects generally associated with corticosteroids may be induced. These include Cushingoid manifestations and muscle weakness. However, sodium and potassium retention, edema, weight gain, psychic aberration and hypertension are exceedingly rare. Dosage should be individualized and kept at the lowest level needed to control symptoms. It should not exceed 36 mg. daily without potassium supplementation. Drug should not be withdrawn abruptly. Contraindicated in herpes simplex and chicken pox.

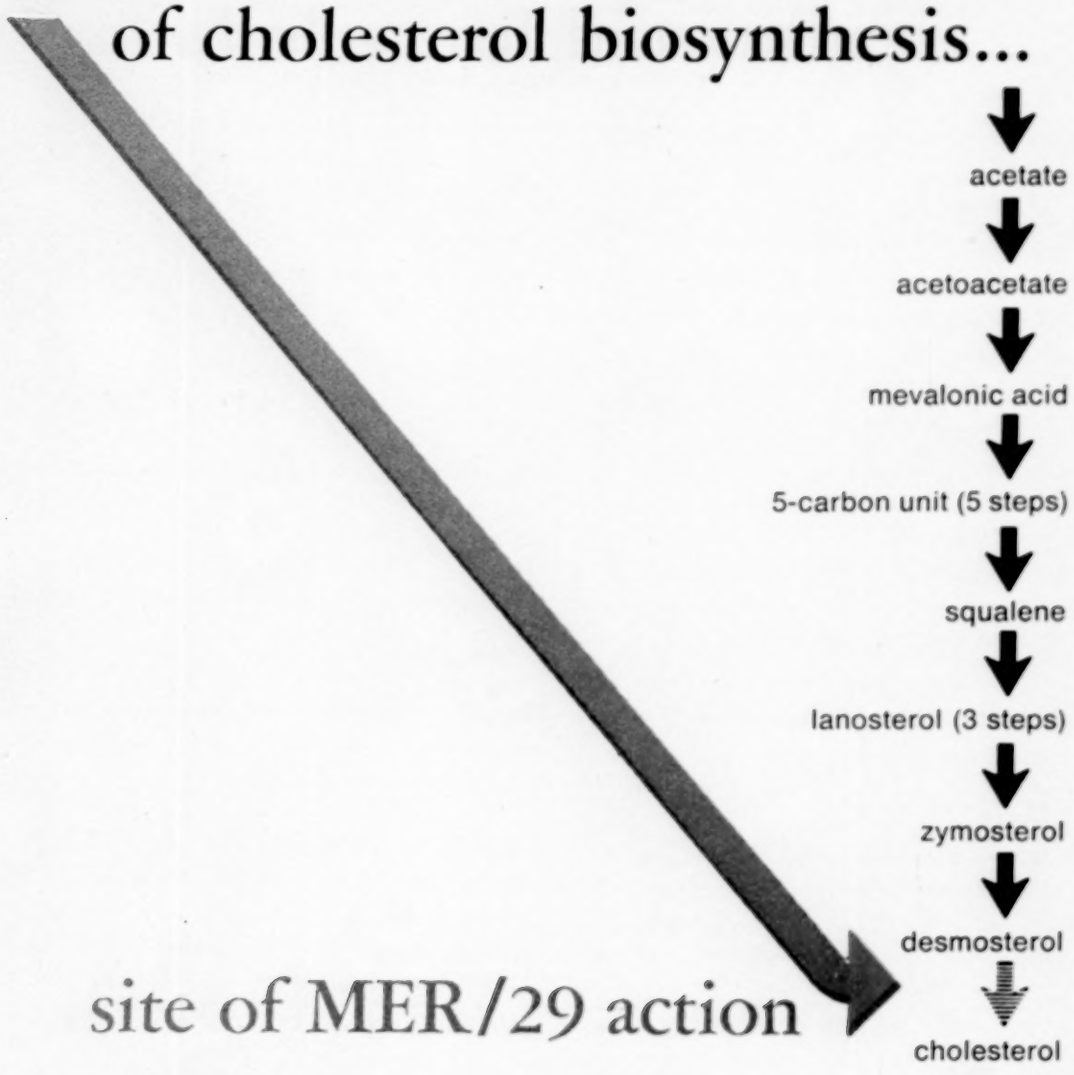
Supplied: Scored tablets — 1 mg. (yellow); 2 mg. (pink); 4 mg. (white); 16 mg. (white).

Lederle

Request complete information on indications, dosage, precautions and contraindications from your Lederle representative or write to Medical Advisory Department.

LEDERLE LABORATORIES, A Division of AMERICAN CYANAMID COMPANY, Pearl River, New York

specific,[1] demonstrated[2] inhibition[3] of cholesterol biosynthesis...



1. The primary, the *only* known action of MER/29 is to lower the total body pool of sterols (serum and tissue); no effect on any other system or organ reported to date.

2. "Using each patient as his own control, the peak *total* sterol radioactivity after injection of mevalonic acid-2-C^{14} was compared on and off MER/29. As much as a 50 per cent inhibition on MER/29 was observed in some patients."
—Steinberg, D.; Avigan, J., and Feigelson, E. B.: Circulation *22*:663 (Oct.) 1960.

3. "Studies of lipid metabolism have stressed the importance of cholesterol biosynthesis, as opposed to cholesterol intake, in determining cholesterol balance."
—National Heart Institute: Diet, Hormones, and Atherosclerosis..., Bethesda, Md., U.S. National Institutes of Health, 1958.

...leading to specific, demonstrated advantages in cholesterol-lowering therapy

particularly in patients with coronary artery disease, generalized atherosclerosis, and other conditions thought to be associated with abnormal cholesterol metabolism

MER/29 REDUCES CHOLESTEROL IN AS MANY AS 8 OUT OF 10 PATIENTS: MER/29 reduces both serum and tissue cholesterol without strict adherence to diet. Although some physicians prefer to use MER/29 in conjunction with controlled diets, cholesterol can be reduced successfully without such limitation.

CONCURRENT BENEFITS REPORTED IN SOME PATIENTS: In patients with coronary artery disease, some of the concurrent benefits reported include decreased incidence and severity of anginal attacks, improved ECG patterns, diminished nitroglycerin dependence, and increased sense of well-being.

MER/29 HAS PRODUCED FEW SIDE EFFECTS, NO TOXICITY: Patients have been treated with MER/29 for continuous periods up to 19 months. In no case has there been evidence of serious toxic effects on the function of any vital organ or system. Side effects (nausea, headache, dermatitis) are rare and have usually been associated with dosages greater than those recommended for effective therapy.

MER/29 is compatible with other cardiovascular therapies. It can be used along with measures which control anxiety, hypertension, obesity and other conditions associated with cardiovascular disorders. These include nitroglycerin, PETN, and anticoagulants.

CAUTION: Since long-term MER/29 therapy may be necessary, periodic examinations, including liver function tests, are desirable. Also, since MER/29 inhibits cholesterol biosynthesis, and cholesterol plays an important role in the development of the fetus, the drug is *contraindicated in pregnancy.*

DOSAGE: One 250 mg. capsule daily, before breakfast.

SUPPLIED: Bottles of 30 pearl gray capsules.

Complete bibliography and product information available on request.

MER/29

(triparanol)

Merrell

The Wm. S. Merrell Company
Division of Richardson-Merrell Inc.
Cincinnati, Ohio • Weston, Ontario

Trademark: MER/29®

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brand of oxyphenbutazone

a new development in nonhormonal, anti-inflammatory therapy

more specific than steroids—
Acts directly on the inflammatory lesion **without** altering pituitary-adrenal function . . . **without** impairing immunity responses.[6-11]

more dependable than enzymes—
Rapid and complete absorption, without the uncertainty of oral or buccal enzyme therapy.[5]

more potent than salicylates—
Anti-inflammatory potency of Tandearil markedly superior to aspirin.[12]

Remarkably useful in a wide variety of inflammatory conditions, including: rheumatoid arthritis, spondylitis, osteoarthritis[1,2,3]; gout[1,4,5]; acute superficial thrombophlebitis[6,7]; painful shoulder (peritendinitis, capsulitis, bursitis, and acute arthritis of that joint)[1-4]; severe forms of a variety of local inflammatory conditions[8,9,10].

The physician should be thoroughly familiar with the dosage, side effects, precautions and contraindications of Tandearil before prescribing. Full product information available on request.

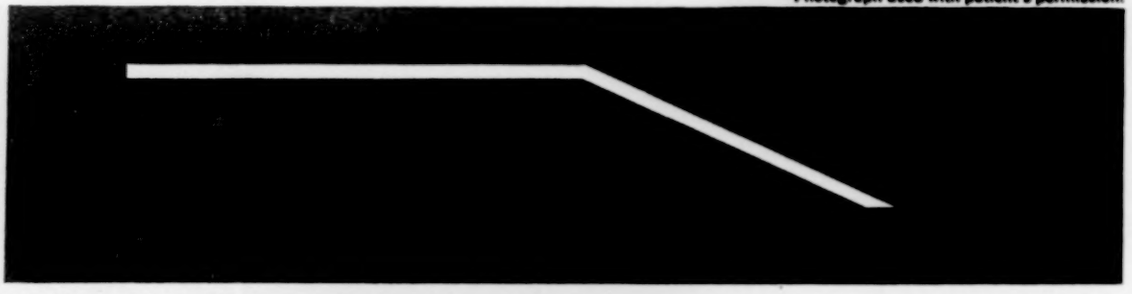
Weight problem? Start the reducing program right, keep it going right with Esidrix® Esidrix-K®

Photograph used with patient's permission.

Recent studies show that the diuretic action of Esidrix improves results of weight-reducing programs 2 ways:

1. **As an adjuvant in initiating treatment:** Esidrix induces greater weight losses in the first few days than a conventional regimen.[1] This weight loss may be significant in itself (depending on the degree of fluid retention). But more than that, the quick loss of even a few pounds builds confidence in the weight-reducing program, inspires determination to follow it faithfully.

2. **As an adjuvant in maintenance treatment:** Esidrix eliminates retained water—with consequent weight losses—to break through the weight plateaus so often encountered in antiobesity programs. (See schematic graph below.) The new weight loss cheers the patient and helps overcome his tendency to eat too much.



(Adapted from Einhorn and Kalb[2])

Esidrix

For complete information about Esidrix and Esidrix-K (including dosage, side effects, and cautions), see Physicians' Desk Reference, or write CIBA, Summit, N. J.

References: 1. Ray, R. E.: To be published. 2. Einhorn, H. P., and Kalb, S. W.: Clin. Med. 7:1995 (Oct.) 1960.

Supplied: ESIDRIX Tablets, 25 mg. (pink, scored) and 50 mg. (yellow, scored). ESIDRIX-K Tablets 25/500 (white, coated), each containing 25 mg. Esidrix and 500 mg. potassium chloride. **NEW STRENGTH ESIDRIX-K NOW AVAILABLE:** ESIDRIX-K Tablets 50/1000 (white, coated), each containing 50 mg. Esidrix and 1000 mg. potassium chloride.

CIBA

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objective:

full term fetus

complication:

threatened abortion

indicated:

Provera

Upjohn

Here are five reasons why:

- Provera is the only commercially-available oral progestational agent that will maintain pregnancy in critical tests in ovariectomized animals.
- It is four times as potent (by castrate assay) as any other progestational agent.
- No significant side effects have been encountered.
- It is available for both oral and parenteral administration
- Provera gives the economy of effective action from small doses.

Brief Basic Information

	● Oral Provera*	I.M. Depo-Provera**
Description	Upjohn brand of medroxyprogesterone acetate.	Aqueous suspension, 50 mg. Provera per cc., for intramuscular injection only.
Indications	Threatened and habitual abortion, infertility, dysmenorrhea, secondary amenorrhea, premenstrual tension, functional uterine bleeding.	Threatened and habitual abortion, endometriosis.
Dosage Threatened abortion	10 to 30 mg. daily until acute symptoms subside.	50 mg. I. M. daily while symptoms are present, followed by 50 mg. weekly through 1st trimester, or until fetal viability is evident.
Habitual abortion 1st trim.	10 mg. daily.	50 mg. I.M. weekly.
2nd trim.	20 mg. daily.	100 mg. I.M. q. 2 wks.
3rd trim.	40 mg. daily, through 8th month.	100 mg. I.M. q. 2 wks. through 8th month.
Supplied:	2.5 mg. scored, pink tablets, bottles of 25; 10 mg. scored, white tablets, bottles of 25 and 100.	Sterile aqueous suspension for intramuscular use only. 50 mg. per cc., in 1 cc. and 5 cc. vials.†

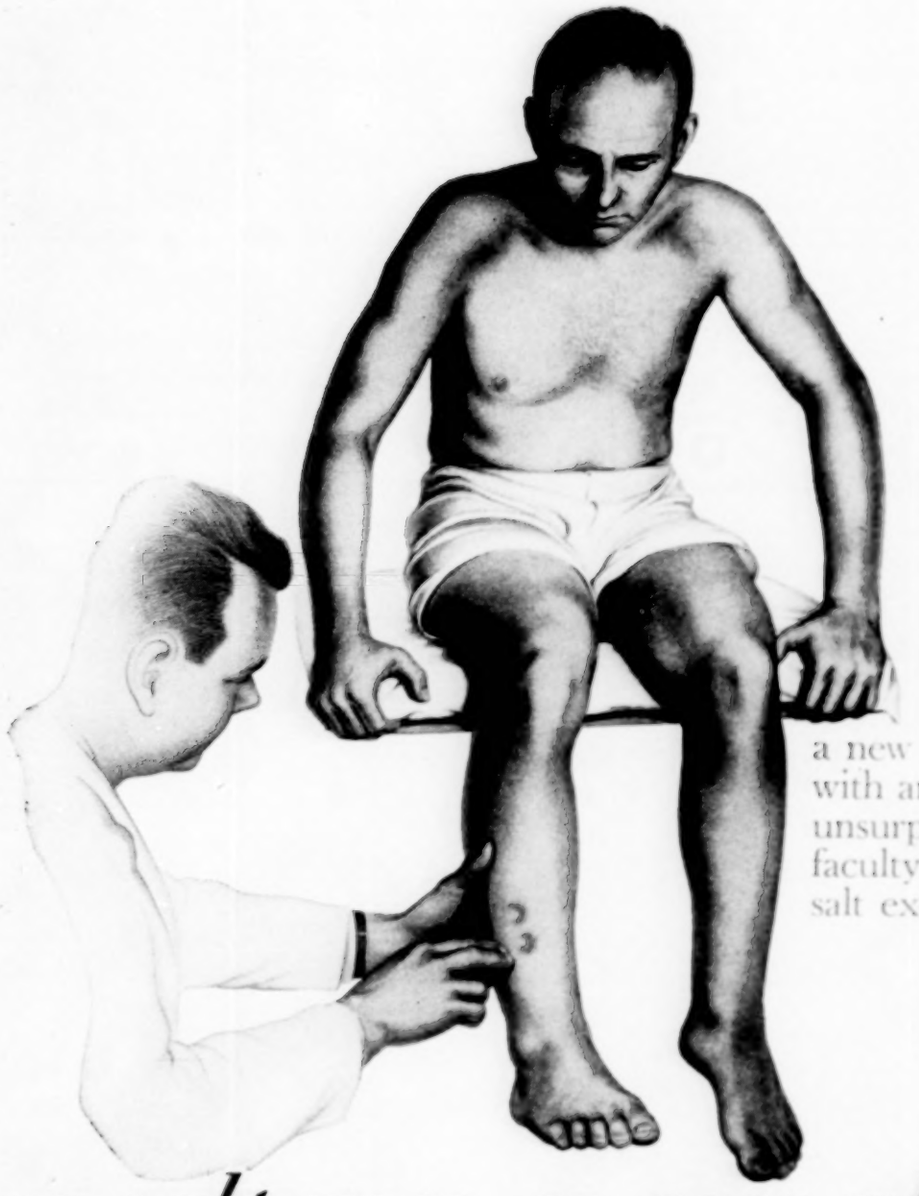
Precautions: Clinically, Provera is well tolerated. No significant untoward effects have been reported. Animal studies show that Provera possesses adrenocorticoid-like activity. While such adrenocorticoid action has not been observed in human subjects, patients receiving large doses of Provera continuously for prolonged periods should be observed closely. Likewise, large doses of Provera have been found to produce some instances of female fetal masculinization in animals. Although this has not occurred in human beings, the possibility of such an effect, particularly with large doses over a long period of time, should be considered.

Provera, administered alone or in combination with estrogens, should not be employed in patients with abnormal uterine bleeding until a definite diagnosis has been established and the possibility of genital malignancy has been eliminated.

†Each cc. of Depo-Provera contains: Medroxyprogesterone acetate, 50 mg.; Polyethylene glycol 4000, 28.8 mg.; Polysorbate 80, 1.92 mg.; Sodium chloride, 8.65 mg.; Methylparaben, 1.73 mg.; Propylparaben, 0.19 mg.; Water for injection, q.s.

The Upjohn Company, Kalamazoo, Michigan

*TRADEMARK, REG. U. S. PAT. OFF. **TRADEMARK



a new diuretic with an unsurpassed faculty for salt excretion

as salt goes, so goes edema

Robins' new NaClex is a potent, oral, non-mercurial diuretic that reduces edema by applying the basic principle that "increased urine volume and loss of body weight are proportional to and the osmotic consequences of loss of ions."[1] NaClex limits the reabsorption of sodium and chloride ions in the renal proximal tubules with a relative sparing of potassium. The body's homeostatic mechanism responds by increasing the excretion of excess extracellular water. Thus the NaClex-induced removal of salt leads to a reduction of edema.

a unique chemical structure:

NaClex (benzthiazide) is a new molecule which provides a "pronounced increase in diuretic potency"[2] over its antecedent sulfonamide compound. On a practical, clinical basis it is unsurpassed in diuretic potency.

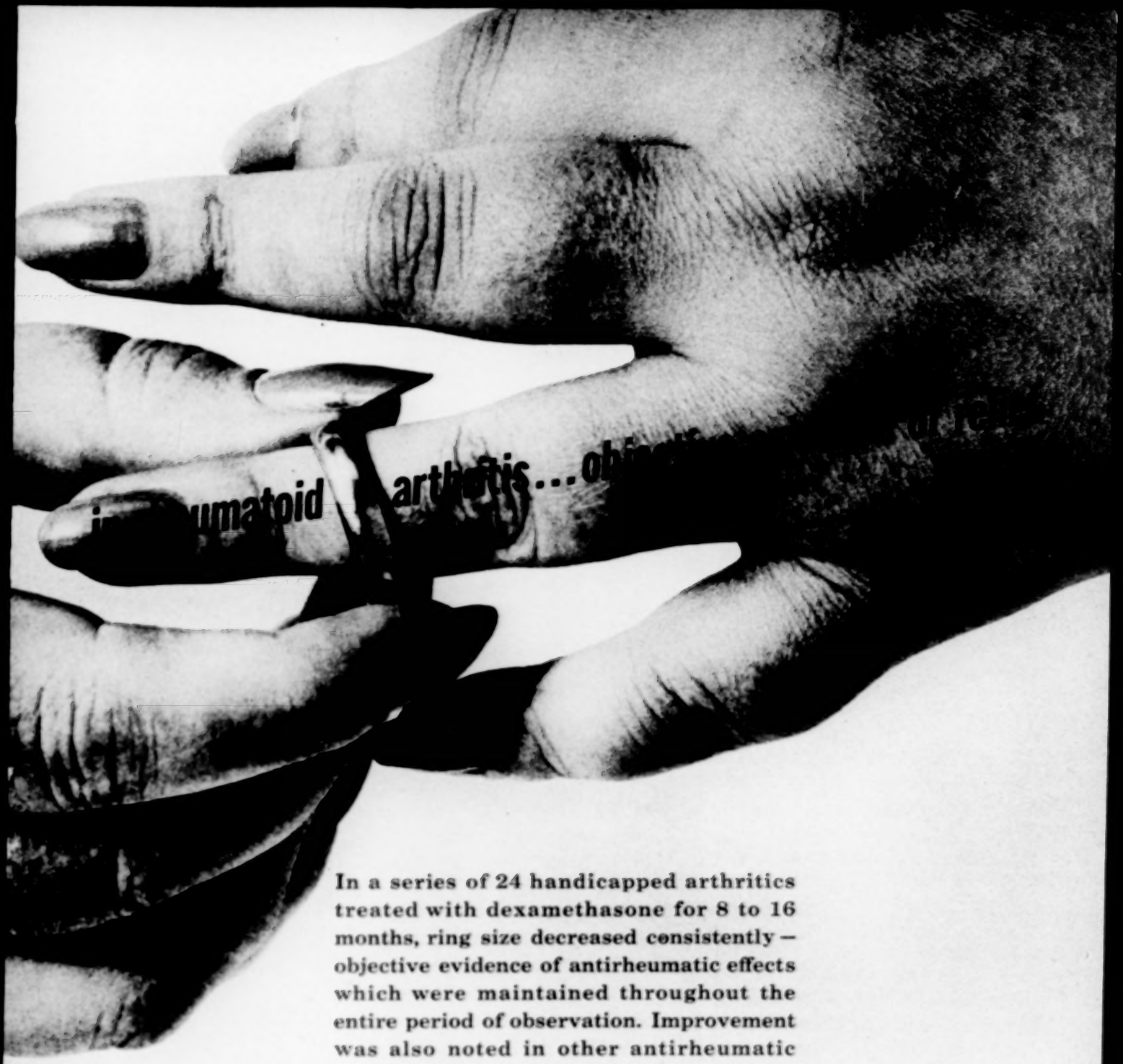
NaClex produces diuresis, weight loss, and symptomatic improvement in edema associated with various conditions. It also has antihypertensive properties and may be used alone in mild hypertension or with other antihypertensive drugs in severer cases.

Available in 50 mg. tablets. Literature on request. Sold in Canada under the tradename EXNA. 1. Pitts, R. F., Am. J. Med., 24:745, 1958. 2. Ford, R. V., Cur. Ther. Res., 2:51, 1960.

A. H. ROBINS CO., INC., RICHMOND, 20, VA.

NaClex benzthiazide®

RX-11B



In a series of 24 handicapped arthritics treated with dexamethasone for 8 to 16 months, ring size decreased consistently—objective evidence of antirheumatic effects which were maintained throughout the entire period of observation. Improvement was also noted in other antirheumatic indices, i. e., pain on motion, tenderness, swelling and morning stiffness.[1]

Supplied: as 0.75 mg. and 0.5 mg. scored, pentagon-shaped tablets in bottles of 100. Also available as Injection DECADRON Phosphate and new Elixir DECADRON. Additional information on DECADRON is available to physicians on request. DECADRON is a trademark of Merck & Co., Inc.

Reference: 1. Bunim, J. J., in Hollander, J. L.: Arthritis and Allied Conditions, ed. 6, Philadelphia, Lea & Febiger, 1960, p. 364.

MERCK SHARP & DOHME
Division of Merck & Co., INC., West Point, Pa.

Decadron®

Dexamethasone

TREATS MORE PATIENTS MORE EFFECTIVELY

A "NEST EGG" HELPS PROVIDE A **SECURE** FUTURE...

ELDEC®
KAPSEALS®

HELP PROVIDE A **HEALTHY** ONE

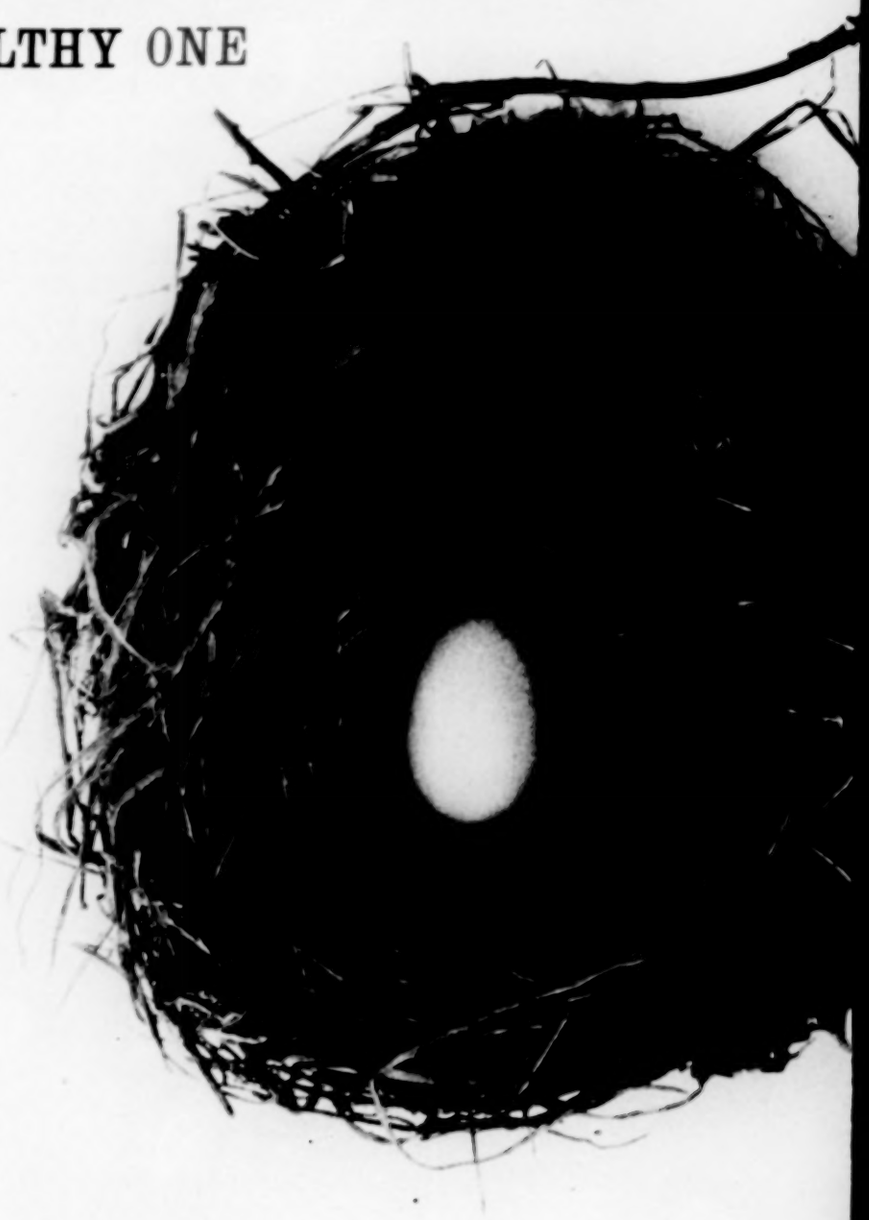
by supplying a dependable source of vitamins, minerals, hormones, digestive enzymes, and amino acids.

Each ELDEC Kapseal contains vitamins—1667 units A, 0.67 mg. B_1 mononitrate, 0.67 mg. B_2, 0.5 mg. pyridoxine hydrochloride, 0.033 N.F. Unit (Oral) B_{12} with intrinsic factor concentrate, 0.1 mg. folic acid, 33.3 mg. C, 16.7 mg. nicotinamide, 10 mg. *dl*-panthenol, 6.67 mg. choline bitartrate; minerals—16.7 mg. ferrous sulfate (exsiccated), 0.05 mg. iodine (as potassium iodide), 66.7 mg. calcium carbonate; digestive enzymes—20 mg. Taka-Diastase® (Aspergillus oryzae enzymes), 133.3 mg. pancreatin; amino acids—66.7 mg. *l*-lysine monohydrochloride, 16.7 mg. *dl*-methionine; gonadal hormones—1.67 mg. methyltestosterone, 0.167 mg. Theelin.

Dosage: One Kapseal three times daily before meals. Female patients should follow each 21-day course with a 7-day rest interval.

Precautions: Contraindicated in patients wherein estrogen or androgen therapy should not be used, as in carcinoma of the breast, genital tract, or prostate, and in patients with a familial tendency to these types of malignancy; give cautiously to females who tend to develop excessive hair growth or other signs of masculinization.

Packaging: ELDEC Kapseals are available in bottles of 100.



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PARKE, DAVIS & COMPANY, Detroit 32, Michigan

Advertisers' Product Index

May, 1961

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*** Complete description of starred drugs will be found in MODERN DRUGS and THE MODERN DRUG ENCYCLOPEDIA.**

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a pair of cardiac patients:

both are free of pain—but only one is on

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swift, sure analgesia normally unmarred by nausea and vomiting

DILAUDID provides unexcelled analgesia in acute cardiovascular conditions. Onset of relief from pain is almost immediate. The high therapeutic ratio of DILAUDID is commonly reflected by lack of nausea and vomiting—and marked freedom from other side-effects such as dizziness and somnolence.

▲ **by mouth** ▲ **by needle** ▲ **by rectum**

2 mg., 3 mg., and 4 mg.

May be habit forming—usual precautions should be observed as with other opiate analgesics.

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Protects the angina patient better than vasodilators alone

Unless the coronary patient's ever-present anxiety about his condition can be controlled, it can easily induce an anginal attack or, in cases of myocardial infarction, can delay recovery.

This is why Miltrate gives better protection for the heart than vasodilators alone in coronary insufficiency, angina pectoris and postmyocardial infarction.

Miltrate contains PETN (pentaerythritol tetranitrate), acknowledged as basic therapy for long-acting vasodilation. . . .

What is more important—Miltrate provides Miltown, a tranquilizer which, unlike phenobarbital, relieves tension in the apprehensive angina patient without inducing daytime fogginess.

Thus, your patient's cardiac reserve is protected against his fear and concern about his condition; his operative arteries are dilated to enhance myocardial blood supply—and he can carry on normal activities more effectively since his mental acuity is unimpaired by barbiturates.

REFERENCES: **1.** Ellis, L. B. *et al.*: Circulation *17*:945, May 1958. **2.** Friedlander, H. S.: Am. J. Cardiol. *1*:395, Mar. 1958. **3.** Riseman, J.E.F.: New England J. Med. *261*:1017, Nov. 12, 1959. **4.** Russek, H. I. *et al.*: Circulation *12*:169, Aug. 1955. **5.** Russek, H. I.: Am. J. Cardiol. *3*:547, April 1959. **6.** Tortora, A. R.: Delaware M. J. *30*:298, Oct. 1958. **7.** Waldman, S. and Pelner, L.: Am. Pract. & Digest Treat. *8*:1075, July 1957.

Supplied: Bottles of 50 tablets. Each tablet contains 200 mg. Miltown and 10 mg. pentaerythritol tetranitrate.

Dosage: 1 or 2 tablets q.i.d. *before meals* and at bedtime, according to individual requirements.

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Miltown® (meprobamate) + PETN

WALLACE LABORATORIES / *Cranbury, N. J.*

In skeletal muscle spasm

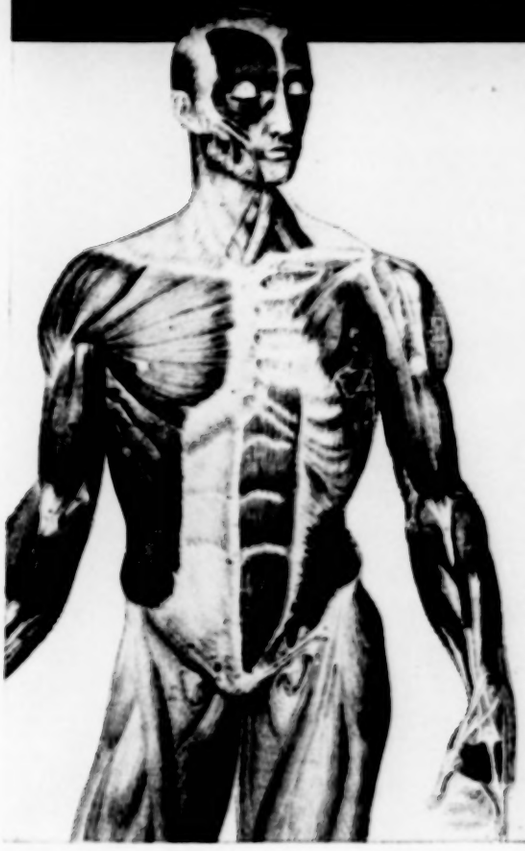
NORFLEX

orphenadrine citrate 100 mg. tablets

quickly resolves the spasm . . .

relieves the pain . . .

restores normal function



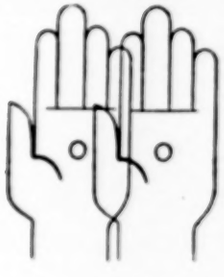
Prolonged relief

may last up to 12 hours after administration . . . permits uninterrupted sleep at night . . . does not interfere with daytime alertness . . . only the muscles in spasm respond . . . no lessening of general muscle tonus.

Contraindications:

Routine precautions against use of anticholinergic drugs should be observed. Norflex should be used with caution in glaucoma, tachycardia, or urinary retention.

Simple dosage



for all adults regardless of age or sex: 2 tablets daily—one in the morning, one in the evening—easily remembered . . . offers better patient cooperation.

NORFLEX is a product of

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*U.S. Patent No. 2,567,361; other patents pending

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How new **Dianabol** rebuilt muscle tissue in this underweight, convalescent patient

Patient was weak and emaciated before Dianabol. R. C., age 51, weighed 160 pounds following surgery to close a perforated duodenal ulcer. His convalescence was slow and stormy, complicated by pneumonia of both lower lobes. Weak and washed out, he was considered a poor risk for further necessary surgery (cholecystectomy). Because a conventional low-fat diet and multiple-vitamin therapy failed to build up R. C. sufficiently, his physician prescribed Dianabol.

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Dianabol: new, low-cost anabolic agent

By promoting protein anabolism, Dianabol builds lean tissue and restores vigor in underweight, debilitated, and dispirited patients. In patients with osteoporosis Dianabol often relieves pain and increases mobility.

As an anabolic agent, Dianabol has been proved 10 times as effective as methyltestosterone. Yet it has far less androgenicity than testosterone propionate, methyltestosterone, or norethandrolone.

Because it is an oral preparation, Dianabol spares patients the inconvenience and discomfort of parenteral drugs.

And because Dianabol is low in cost, it is particularly suitable for the aged or chronically ill patient who may require long-term anabolic therapy.

Supplied: *Tablets*, 5 mg. (pink, scored); bottles of 100.

Dianabol®

(methandrostenolone CIBA)

converts protein to working weight in wasting or debilitated patients

For complete information about Dianabol (including dosage, cautions, and side effects), see Physicians' Desk Reference or write CIBA, Summit, N. J.

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Nardil relieves the anxiety by removing the hidden depression

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High Tolerance + High Potency
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CAPLETS®

Iron
can
be
pleasant

Fergon Plus provides ferrous gluconate in a nonastringent highly soluble form for rapid absorption, rapid hemoglobin response and maximum gastrointestinal tolerance. Reported clinical findings[1-4] stress the relative absence of nausea, constipation, diarrhea or abdominal cramps.

Each Fergon Plus Caplet contains:

Fergon (brand of ferrous gluconate)..500 mg.
Iron without Irritation

Vitamin B_{12} with intrinsic factor concentrate N.F.....½ unit (oral)

Ascorbic acid ..... 75 mg.

HOW SUPPLIED:

Fergon Plus Caplets, bottles of 100 and 500.

The "Plus" Factors in Fergon Plus

Fergon Plus contains ascorbic acid to help assure maximum absorption and utilization of iron as well as other essential factors to aid in hemopoiesis of iron deficiency and most macrocytic anemias.

To obtain better tolerance and quicker response to therapy and to insure proper maintenance—

prescribe **FERGON PLUS**

DOSAGE:

Therapeutic — 2 Caplets daily (one before the morning and evening meals).

As dietary supplement—1 Caplet daily.

also available

FERGON C C CAPLETS

Each sugar-coated Caplet contains 450 mg. of ferrous gluconate (yielding 50 mg. of elemental iron) and 200 mg. of ascorbic acid.

Prolonged vitamin C deficiency may lead to hypochromic anemia. Vitamin C aids in maximum utilization of iron. *Bottles of 100.*

References:
1. Haler, David: *Lancet* 2:1018, Nov. 13, 1954. 2. Wagley, P. F.: *Maryland M.J.* 2:351, July, 1953. 3. Jones, W. M.: *Brit. M.J.* 1:1050, May 9, 1953. 4. Jones, W. M.: *Practitioner* 170:185, Feb., 1953.

Winthrop LABORATORIES
New York 18, N.Y.

The American Journal of Medicine

Vol. XXX JUNE 1961 No. 6

CONTENTS

Editorial

Clinical Studies

Dr. Huckabee has been interested in blood lactate concentrations for some time and here summarizes certain of his observations. The first paper gives the normal range of values, and describes moderate elevations sporadically encountered in nondescript diseases, sometimes of definable cause, at other times of speculative origin. One small group of nine patients stood out, however, and is described in the second paper. These patients all had marked acidosis due to lactic acid alone; accompanying this there were the usual respiratory manifestations of acidosis and progressive deterioration to death. This seems to be a distinct syndrome, recognizable only by the excessive blood lactate concentration, of obscure cause and poor prognosis.

This study demonstrates that periodic breathing is a pattern of neurogenic hyperpnea in which hyperventilation alternates with posthyperventilation apnea. Patients with Cheyne-Stokes respiration have bilateral supramedullary brain dysfunction resulting in increased respiratory sensitivity to CO_2 and therefore in hyperpnea. Ventilation is further augmented by reduced arterial oxygen tensions resulting from moderate arterial unsaturation together with alkalosis. For this reason, anoxemia continues to drive ventilation during late respiratory decrescendo despite $PaCO_2$ levels below the respiratory stimulating threshold.

The arterial O_2 saturation, arterial pCO_2, cerebral A-V O_2 difference, cerebral circulation time and spinal fluid pressure were measured in patients with Cheyne-Stokes respiration. Encephalograms also were obtained. Correlation of the data led to the conclusion that Cheyne-Stokes respiration is only the most obvious manifestation of a more complex syndrome which includes phasic circulatory, mental and neurogenic changes in addition to the periodic breathing.

Contents continued on page 5

Stop the pain in minutes

When the infection is accompanied by pain, burning or frequency, phenylazo-diamino-pyridine HCl, the local analgesic component, soothes the inflamed urinary mucous membranes. Relief usually comes within a half hour after administration.

Control urinary pathogens

Gantrisin proves effective in most bacterial infections of the genitourinary tract, whether carried by the blood stream or urine. Safety is assured through high solubility.

Gantrisin—"The Quality of Greatness"

Composition: Each tablet contains 500 mg of Gantrisin plus 50 mg of phenylazo-diamino-pyridine HCl. **Usual Adult Dosage:** 2 tablets, 4 times daily. **Warning:** The usual precautions in sulfonamide therapy should be observed. If toxic reactions or blood dyscrasias occur, discontinue administration of the drug. Because Azo Gantrisin contains phenylazo-diamino-pyridine hydrochloride, it is contraindicated in glomerular nephritis, severe hepatitis and uremia. In such cases, Gantrisin should be used alone.

GANTRISIN®—brand of sulfisoxazole

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VOLUME THIRTY NUMBER SIX

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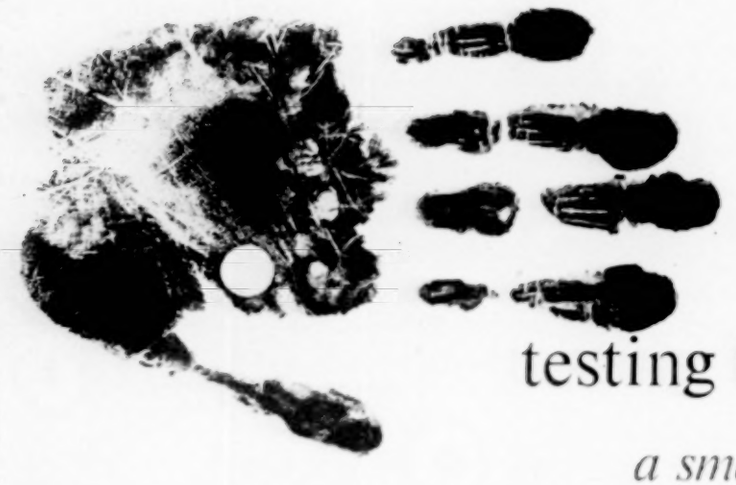

AN AMES CLINIQUICK®
CLINICAL BRIEFS FOR MODERN PRACTICE

testing for ketonuria

a small extra premium to insure oral control of diabetes

Freedom from *both* glucosuria *and* ketonuria is a basic criterion of successful oral treatment of diabetes.[1] During the first week of treatment the patient should test his urine four times daily for sugar and ketones and should report several times to the physician.[2]

Subsequent oral control of diabetes is also stabilized by routine testing for ketonuria. A positive finding may warn of impending drug failure caused by development of tolerance or intercurrent illness.[1] Moreover, the diabetic rate of fat oxidation and ketone synthesis is abnormally high.[3] Inattention to diet may aggravate this abnormality.

Reduction of weight—often the most valuable part of "oral" management of diabetes—is thus a special indication for urine-ketone testing. Increased catabolism of body fat may decrease carbohydrate tolerance, as well as induce ketonemia and acidosis.[4] Too rapid loss of weight may also result in hyperlipemia with a relative preponderance of saturated fatty acids, and thereby tend to alter coagulation, increase atheroma or induce thrombosis.[5]

References: (1) Lee, C. T., Jr., and Duncan, G. G., in Conn, H. F.: Current Therapy 1960, Philadelphia, Saunders, 1960, pp. 287-289. (2) Williams, R. H.: Diabetes, New York, Hoeber, 1960, p. 500. (3) Joslin, E. P., in Joslin, E. P.; Root, H. F.; White, P., and Marble, A.: The Treatment of Diabetes Mellitus, ed. 10, Philadelphia, Lea & Febiger, 1959, pp. 294-295. (4) Siperstein, M. D., in Williams, R. H.: *op. cit.* (ref. 2) pp. 114-117. (5) Beckett, A. G., and Lewis, J. G.: Lancet 2:14 (July 2) 1960.

Your prudent diabetic patient can readily be made to appreciate the value of adding ketone testing with ACETEST to his routine sugar testing with CLINITEST.® He should recognize that the price of this freedom from injections is eternal vigilance against glucosuria *and* ketonuria.

standard "on-the-spot" test for ketonuria

01261

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Elkhart • Indiana
Toronto • Canada

AMES

ACETEST®
BRAND *Reagent Tablets*

"... 1 drop of urine is placed on the tablet. The resultant color is read after 30 seconds."
(Fajans, S. S., in Williams, R. H.: *op. cit.*,[2] p. 420)

also available: **KETOSTIX®**
BRAND *Reagent Strips*
simple "dip-and-read" test for ketonuria

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Clinicopathologic Conference

Case Reports

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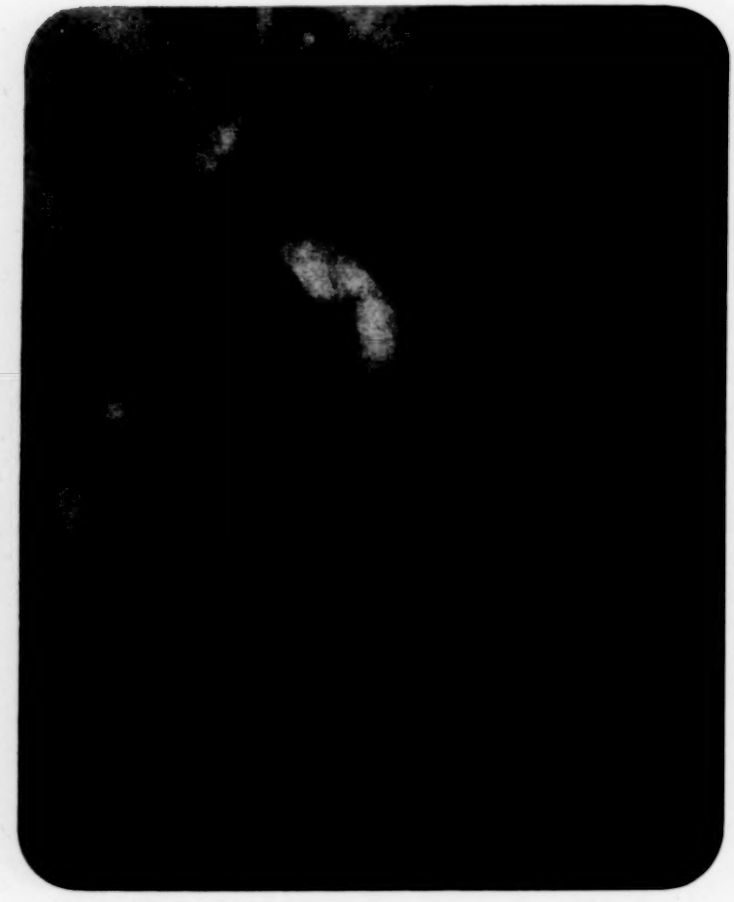
"Outstanding Contribution"[1] in Cholangiography



Rapid Optimal Opacification: Within 15 minutes, Cholografin outlines hepatic and common ducts even after cholecystectomy, reveals biliary ducts in about 25 minutes, and completely opacifies the gallbladder within 2½ hours.[1-4]

High Diagnostic Accuracy: Accuracy in diagnosing bile duct disease in postcholecystectomized patients is reported to be 86%.[1] In another series,[5] Cholografin permitted diagnostic interpretation in almost 70% of patients with chronic cholecystitis.

"With Little Risk"[3] No hepatic or renal toxicity, no delayed reactions have been observed.[3] Cholografin "is the method of choice"[6] for gallbladder visualization in infants.

Cholografin

Sodium / Methylglucamine

Duografin Squibb Diatrizoate and Iodipamide Methylglucamines for rapid visualization of biliary *and* renal tracts in routine examinations or differential diagnosis

Supply: Cholografin Methylglucamine Squibb Iodipamide Methylglucamine Injection U.S.P. is supplied in 20 cc. sizes, with sufficient excess for sensitivity testing.

Cholografin Sodium Squibb Iodipamide Sodium Injection N.F. is supplied in cartons containing two 20 cc. ampuls with sufficient excess for sensitivity testing.

Duografin is supplied in bottles of 50 cc.

For full information, see your Squibb Product Reference or Product Brief.

References: (1) Cohn, E. M.: Am. J. Gastroenterol. *35*:115 (Feb.) 1961. (2) Jones, M. D.; Sakai, H.; and Rogerson, A. G.: J. Pediat. *53*:172 (Aug.) 1958. (3) Machella, T. E.: Gastroenterology *34*:1050 (June) 1958. (4) Orloff, T. L.: Am. J. Roentgenol. *80*:618 (Oct.) 1958. (5) Johnson, G., Jr.; Pearce, C.; and Glenn, F.: Ann. Surg. *152*:91 (July) 1960. (6) McClenahan, J. L.: Pennsylvania M. J. *62*:188 (Feb.) 1959.

SQUIBB

Squibb Quality — the Priceless Ingredient

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Change of address must reach us one month preceding month of issue.

inside as well as outside the hospital... staphylococci usually remain sensitive to

CHLOROMYCETIN®

(chloramphenicol, Parke-Davis)

That the sensitivity patterns of "street" staphylococci differ widely from those of "hospital" staphylococci is a well-established clinical fact.[1-5] Although strains of staphylococci encountered in general practice have remained relatively sensitive to a number of antibiotics,[6] the problem of antibiotic-resistant staphylococci appears to be a threat to all patients in hospitals today. It is encouraging to note, however, "...that a relatively small percentage of strains develop resistance to chloramphenicol, despite the consumption of large amounts of this antibiotic."[7]

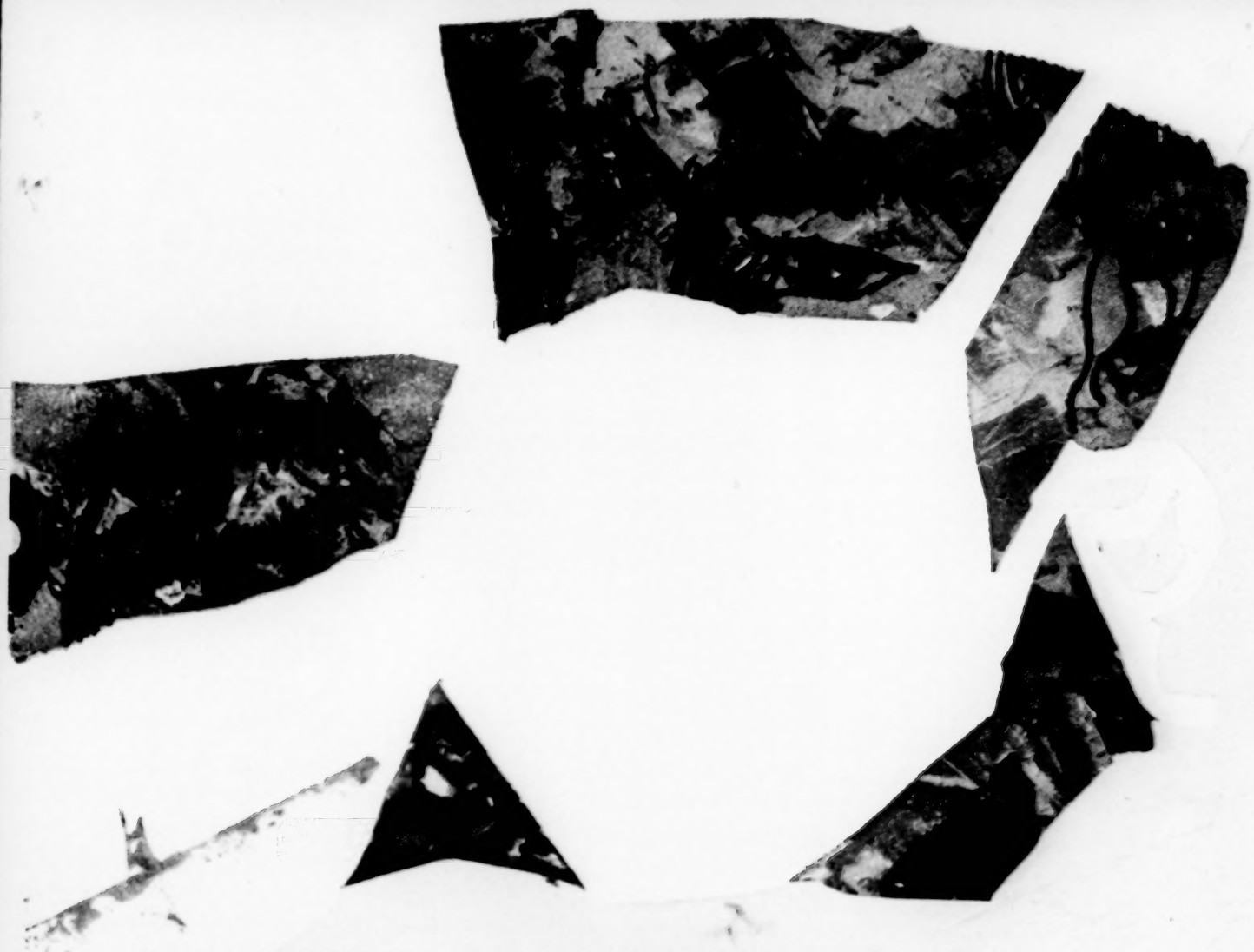
In one hospital, for example, CHLOROMYCETIN "...was the only widely used antibiotic to which few of the strains were resistant."[8] In another hospital, despite steadily increasing use of CHLOROMYCETIN since 1956, "...the percentage of chloramphenicol-resistant strains has actually been lower in subsequent years."[1] Elsewhere, insofar as hospital staphylococci are concerned, it appears that "...the problem of antibiotic resistance can be regarded as minimal for chloramphenicol."[2]

CHLOROMYCETIN (chloramphenicol, Parke-Davis) is available in various forms, including Kapseals® of 250 mg., in bottles of 16 and 100.

See package insert for details of administration and dosage.

Warning: Serious and even fatal blood dyscrasias (aplastic anemia, hypoplastic anemia, thrombocytopenia, granulocytopenia) are known to occur after the administration of chloramphenicol. Blood dyscrasias have occurred after short-term and with prolonged therapy with this drug. Bearing in mind the possibility that such reactions may occur, chloramphenicol should be used only for serious infections caused by organisms which are susceptible to its antibacterial effects. Chloramphenicol should not be used when other less potentially dangerous agents will be effective, or in the treatment of trivial infections such as colds, influenza, viral infections of the throat, or as a prophylactic agent.

Precautions: It is essential that adequate blood studies be made during treatment with the drug. While blood studies may detect early peripheral blood changes such as leukopenia or granulocytopenia, before they become irreversible, such studies cannot be relied upon to detect bone marrow depression prior to development of aplastic anemia.



IN VITRO SENSITIVITY OF 250 STRAINS OF STAPHYLOCOCCI TO CHLOROMYCETIN AND TO FOUR OTHER ANTIBIOTICS*

These strains of coagulase-positive staphylococci were isolated from hospitalized patients at a large county hospital during the year 1959. Sensitivity tests were done by the disc method.

*Adapted from Bauer, Perry, & Kirby[1]

References: (1) Bauer, A. W.; Perry, D. M., & Kirby, W. M. M.: *J.A.M.A.* **173**:475, 1960. (2) Fisher, M. W.: *Arch. Int. Med.* **105**:413, 1960. (3) Cohen, S.: *Circulation* **20**:96, 1959. (4) Edwards, T. S.: *Am. J. Ophth.* **48**, Part II:19, 1959. (5) Smith, I. M.: Staphylococcal Infections, Chicago, The Year Book Publishers, Inc., 1958, p. 148. (6) Petersdorf, R. G.; Rose, M. C.; Minchew, H. B.; Keene, W. R., & Bennett, I. L., Jr.: *Arch. Int. Med.* **105**:398, 1960. (7) Editorial: *J.A.M.A.* **173**:544, 1960. (8) Finland, M.; Jones, W. F., Jr., & Bennett, I. L., Jr.: *Arch. Int. Med.* **104**:365, 1959.

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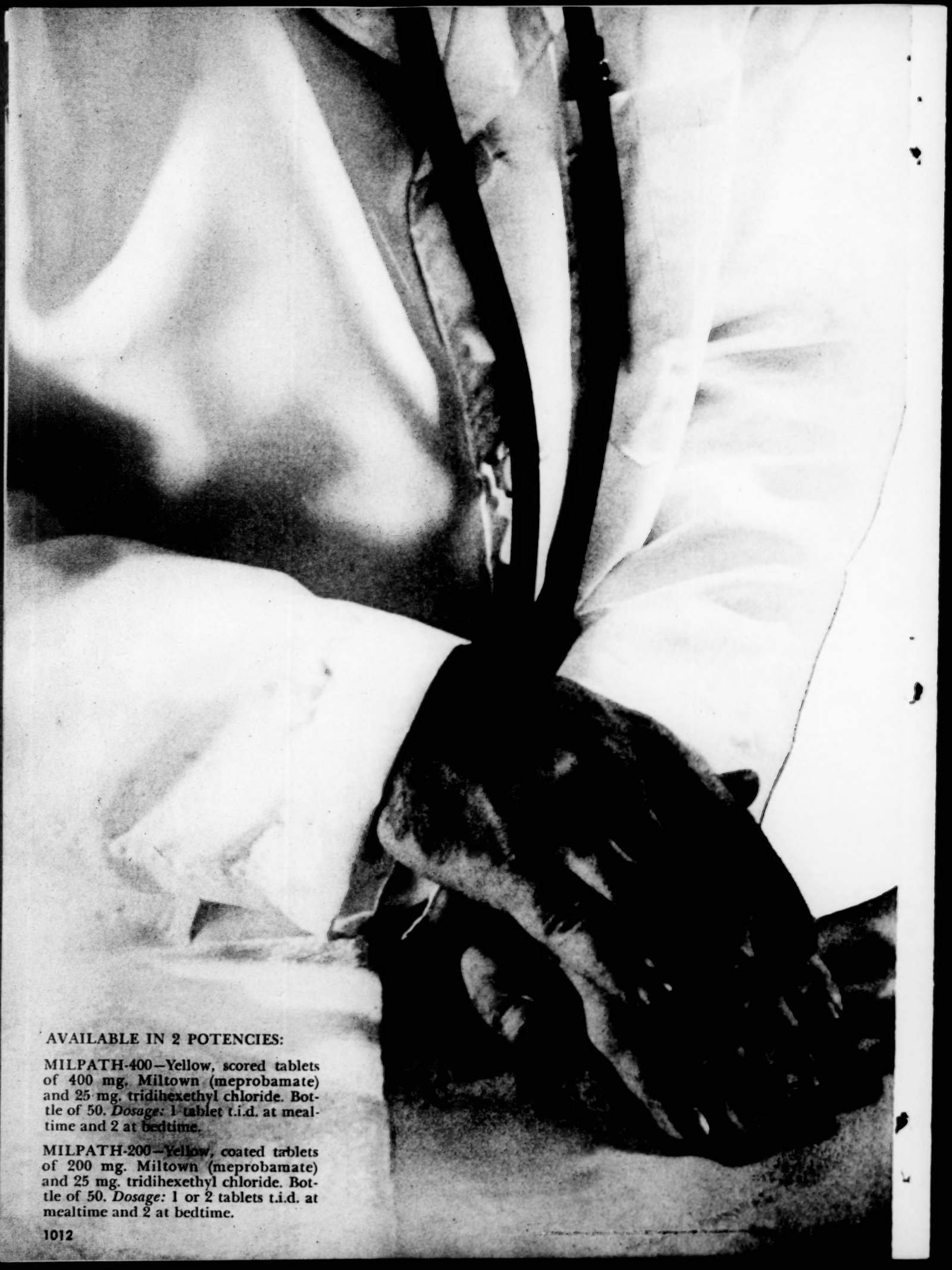
PARKE, DAVIS & COMPANY, Detroit 32, Michigan



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MILPATH-400—Yellow, scored tablets of 400 mg. Miltown (meprobamate) and 25 mg. tridihexethyl chloride. Bottle of 50. *Dosage:* 1 tablet t.i.d. at mealtime and 2 at bedtime.

MILPATH-200—Yellow, coated tablets of 200 mg. Miltown (meprobamate) and 25 mg. tridihexethyl chloride. Bottle of 50. *Dosage:* 1 or 2 tablets t.i.d. at mealtime and 2 at bedtime.

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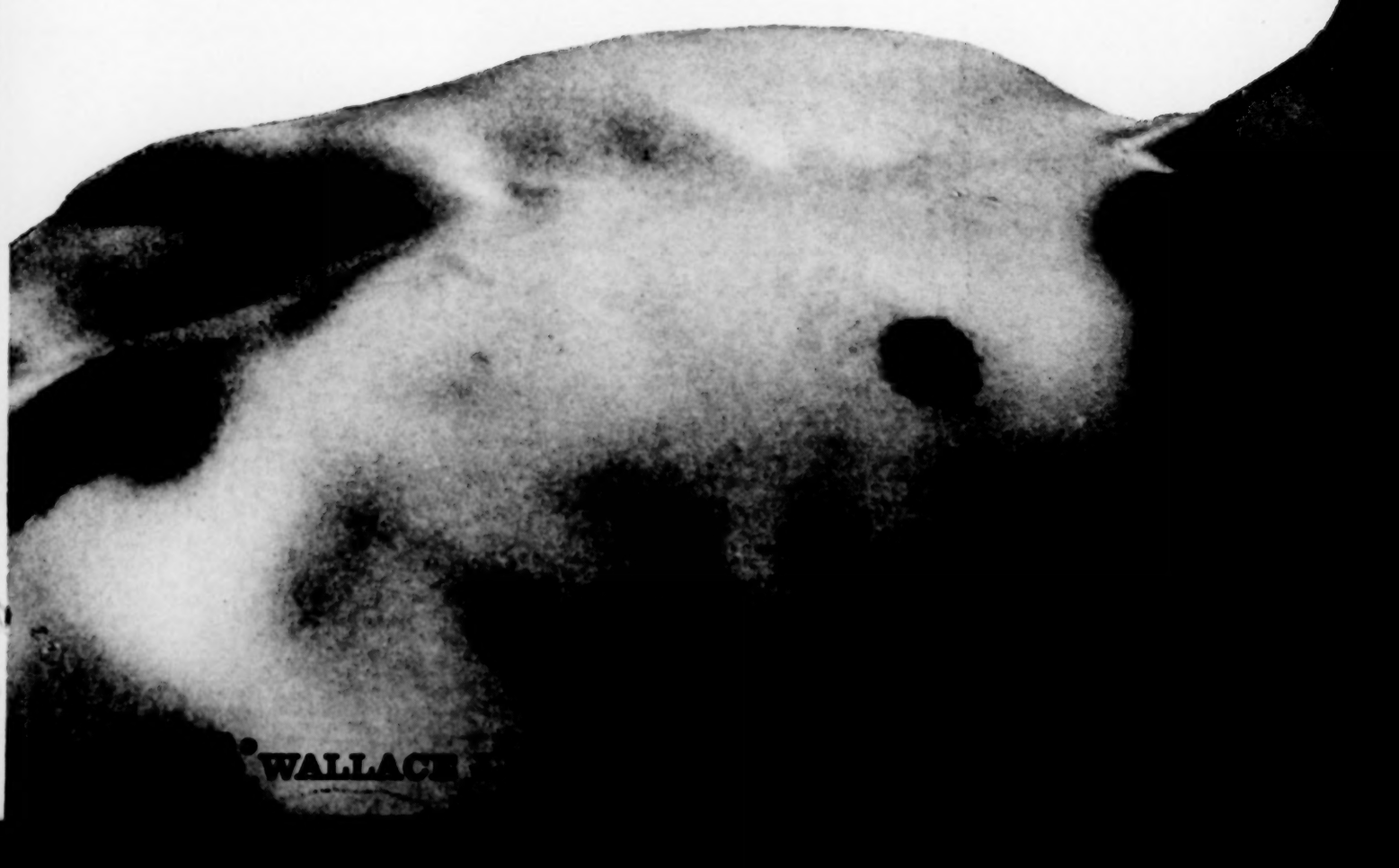
Milpath helps you provide care of the man, rather than merely his stomach:

acts quickly to suppress hypermotility, hypersecretion, spasm and pain . . . alleviate anxiety and tension with minimal side effects.

Milpath®

®Miltown + *anticholinergic*



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Women of menstrual age, many growing children, blood donors, geriatric patients and convalescents may need a hematinic . . . and all can benefit from Livitamin.

Livitamin offers the ideal formula to restore depleted iron reserves and give nutritional support—an important aspect of iron deficiency.

Iron in Livitamin is well absorbed and stored and well tolerated. B complex and other ingredients provide integrated nutritional support.

And Livitamin is a boon for your taste-fussy patients who *should* but *will not* take a hematinic.

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. . . the hematinic with built-in nutritional support

FORMULA: Each fluidounce contains:

Iron peptonized (equiv. in elemental iron to 71 mg.)	420 mg.
Manganese citrate, soluble, N.F.	158 mg.
Thiamine hydrochloride	10 mg.
Riboflavin	10 mg.
Cobalamin	20 mcg.
Nicotinamide	50 mg.
Pyridoxine hydrochloride	1 mg.
Pantothenic acid	5 mg.
Liver fraction 1	2 Gm.
Rice bran extract	1 Gm.
Inositol	30 mg.
Choline	60 mg.

SUPPLIED: Liquid or capsule; also available as capsules, LIVITAMIN with Intrinsic Factor.

THE S. E. MASSENGILL COMPANY

Bristol, Tennessee • New York • Kansas City • San Francisco

NOW WIDELY PRESCRIBED QUINIDINE THERAPY IN CARDIAC ARRHYTHMIAS

Quinaglute T.M. dura-tab® s.m.

exclusive oral
Sustained Medication*
Quinidine Gluconate (5 gr.)

for these good reasons[1-4]

q. 12 h. dosage
QUINAGLUTE DURA-TAB S.M. tablets maintain effective uniform blood levels around the clock, day and night.

better tolerated
because quinidine gluconate is ten times more soluble than the sulfate — and easier on the g.i. tract.

uniformly efficient
no let-down in plasma levels where arrhythmias tend to occur.

PDR
PAGE 821

Bottles of 30, 100 and 250.

1. Bellet, S., Finkelstein, D., and Gilmore, H.: A.M.A. Archives Int. Med. 100:750, 1957.
2. Bellet, S.: Amer. Heart J. 56:479, 1958.
3. Finkelstein, D.: Penn. Med. J. 61:1216, 1958.
4. Bellet, S.: Amer. J. Cardiology 4:268, 1959.

Samples and literature — write . . .

WYNN PHARMACAL CORPORATION
Lancaster Ave. at 51st Street, Philadelphia 31, Pa.

Also available: INJECTABLE QUINAGLUTE
10 cc. Vials, 0.08 Gm. Quinidine Gluconate per cc.

*U.S. Patent 2895881

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June 25–30—New York City

FASHION SAYS PURPLE...DANDRUFF SAYS WHITE! She could just cry, she's so miserable. Purple is the big color this year and it would go so well with her hair—but not with her dandruff. When will she learn that a scalp problem is a medical problem? Probably never... unless you tell her yourself. Why not give her some friendly medical advice...and a prescription for Selsun? You can count on her gratitude just as surely as you can count on good results with Selsun. Remember: Selsun is effective in 95% of your patients with seborrheic dermatitis. (And that includes the man in the blue suit in your waiting room right now.)

SELSUN®
SUSPENSION
AN ETHICAL ANSWER TO A MEDICAL PROBLEM
Selsun—Selenium Sulfide, Abbott

2 Iberol Filmtabs a day supply:

The right amount of Iron

Ferrous Sulfate, U.S.P..... 1.05 Gm.
(Elemental Iron—210 mg.)

Plus Therapeutic B-Complex

Cobalamin (Vitamin B_{12}).... 25 mcg.
Liver Fraction 2, N. F..... 200 mg.
Thiamine Mononitrate..... 6 mg.
Riboflavin..... 6 mg.
Nicotinamide..... 30 mg.
Pyridoxine Hydrochloride..... 3 mg.
Calcium Pantothenate..... 6 mg.

Plus Vitamin C

Ascorbic Acid..... 150 mg.

Filmtab—Film-sealed tablets, Abbott

NOTE: Iberol®-F with 1 mg. of Folic Acid in each Filmtab is available on your prescription.

ABBOTT

Anemia of pregnancy

Another indication for Filmtab® IBEROL

(Iron, Vitamin B_{12}, and Other Vitamins, Abbott)

Potent antianemia therapy plus therapeutic B-complex, in the exclusive Filmtab coating which protects stability—locks in vitamin taste and odor.

to combat the three-pronged assault of urinary tract infections *— bacteriuria — tissue infection — discomfort*

UROBIOTIC®

COSA-TERRAMYCIN®—SULFONAMIDE—ANALGESIC

Only UROBIOTIC *contains:* OXYTETRACYCLINE (with glucosamine for enhanced absorption) — notable for its wide tissue distribution, high urinary concentration, excellent toleration and proven antibiotic effectiveness against even so troublesome an invader as *Pseudomonas;* SULFAMETHIZOLE — an unusually soluble, highly active sulfonamide; PHENYLAZO-DIAMINO-PYRIDINE — for effective local analgesia.

IN BRIEF

INGREDIENTS: Each Urobiotic capsule contains 125 mg. Terramycin® (oxytetracycline) with 125 mg. glucosamine HCl, 250 mg. sulfamethizole, and 50 mg. phenylazo-diamino-pyridine HCl.

INDICATIONS: Urobiotic is indicated in the treatment of a number of common genitourinary infections caused by susceptible organisms. It may also be used prophylactically before and after genitourinary or pelvic surgery, following instrumentation procedures, during the use of retention catheters, and in patients with conditions such as cord bladder or cystocele.

DOSAGE: In adults, a dose of 1 or 2 capsules four times daily is suggested, depending upon the severity and response of the infection. In children 60 to 100 lbs., the suggested average dose is 1 capsule four times daily; in children under 60 lbs., 1 capsule three times daily. Therapy should be continued for a minimum of 7 days or until bacteriologic cure is effected in acute urinary tract infections.

CONTRAINDICATIONS: Urobiotic may be contraindicated in patients with chronic glomerulonephritis, hepatitis, hepatic failure, uremia, and obstructive lesions of the urinary tract, and should not be used in patients sensitive to any of its components.

PRECAUTIONS: The use of broad-spectrum antibiotics may, in rare cases, result in an overgrowth of nonsusceptible organisms, such as monilia or staphylococci. Should such superinfection occur, therapy with Urobiotic should be discontinued and specific therapy instituted as shown by susceptibility testing. The use of sulfonamides may cause renal crystalluria or skin rash, as well as other toxic or sensitivity reactions. If any of these occur, discontinue use.

SUPPLIED: Urobiotic capsules, yellow-and-grey, bottles of 50.

More detailed professional information available on request.

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in edema or hypertension

more doctors are prescribing—

more patients are receiving the benefits of—

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than for any other diuretic-antihypertensive

DIURIL is unique. There is no other brand of chlorothiazide.

Dosage: Edema—One or two 500-mg. tablets DIURIL once or twice a day. Hypertension—One 250-mg. tablet DIURIL or one 500-mg. tablet DIURIL two to three times a day.

Supplied: 250-mg. and 500-mg. scored tablets DIURIL chlorothiazide in bottles of 100 and 1000.

DIURIL is a trademark of Merck & Co., INC.

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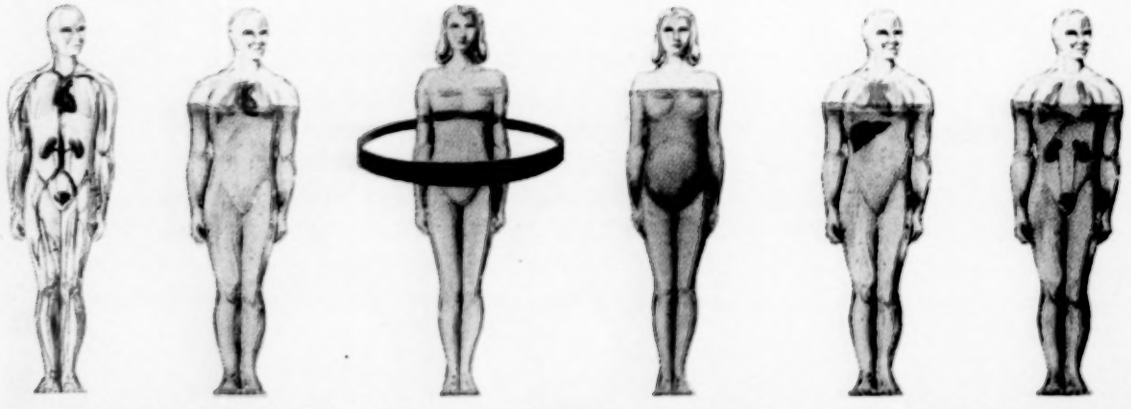
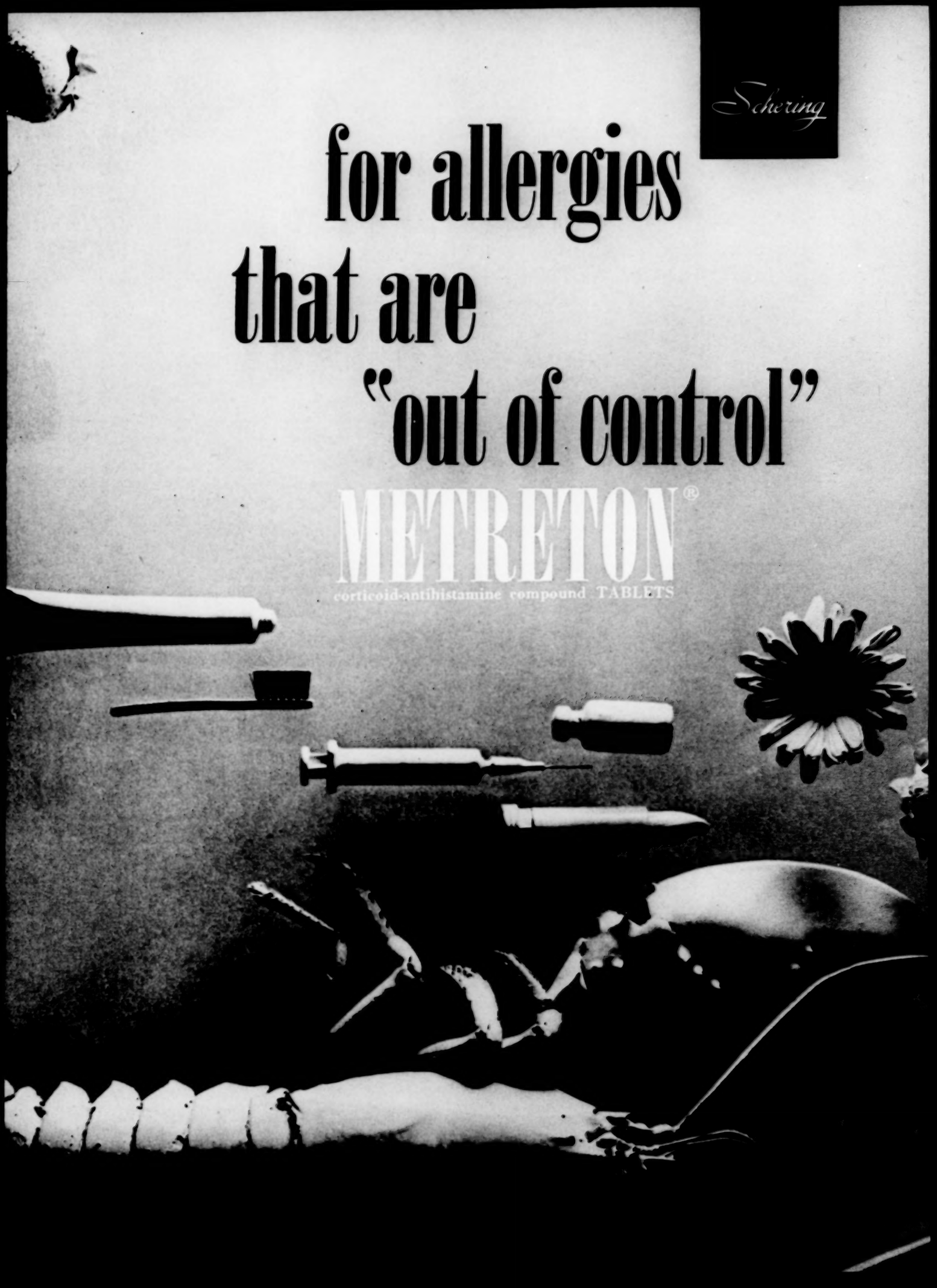


HYPERTENSION CONGESTIVE FAILURE PREMENSTRUAL TENSION EDEMA OF PREGNANCY CIRRHOSIS WITH ASCITES RENAL EDEMA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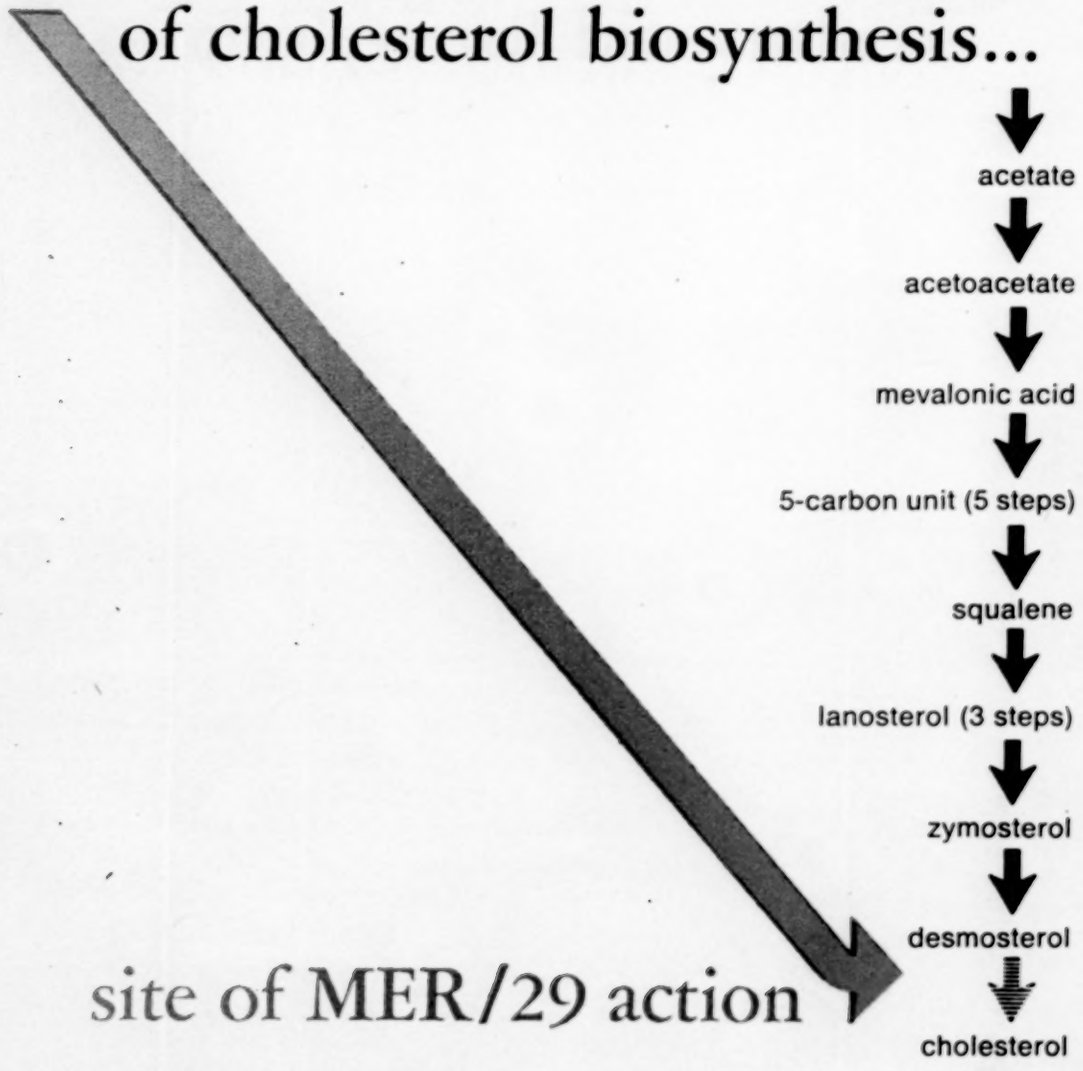
for allergies
that are
"out of control"
METRETON®
corticoid-antihistamine compound TABLETS

specific[1], demonstrated[2] inhibition[3] of cholesterol biosynthesis...



1. The primary, the *only* known action of MER/29 is to lower the total body pool of sterols (serum and tissue); no effect on any other system or organ reported to date.

2. "Using each patient as his own control, the peak *total* sterol radioactivity after injection of mevalonic acid-2-C^{14} was compared on and off MER/29. As much as a 50 per cent inhibition on MER/29 was observed in some patients."
—Steinberg, D.; Avigan, J., and Feigelson, E. B.: Circulation *22*:663 (Oct.) 1960.

3. "Studies of lipid metabolism have stressed the importance of cholesterol biosynthesis, as opposed to cholesterol intake, in determining cholesterol balance."
—National Heart Institute: Diet, Hormones, and Atherosclerosis..., Bethesda, Md., U.S. National Institutes of Health, 1958.

...leading to specific, demonstrated advantages in cholesterol-lowering therapy

particularly in patients with coronary artery disease, generalized atherosclerosis, and other conditions thought to be associated with abnormal cholesterol metabolism

MER/29 REDUCES CHOLESTEROL IN AS MANY AS 8 OUT OF 10 PATIENTS: MER/29 reduces both serum and tissue cholesterol without strict adherence to diet. Although some physicians prefer to use MER/29 in conjunction with controlled diets, cholesterol can be reduced successfully without such limitation.

CONCURRENT BENEFITS REPORTED IN SOME PATIENTS: In patients with coronary artery disease, some of the concurrent benefits reported include decreased incidence and severity of anginal attacks, improved ECG patterns, diminished nitroglycerin dependence, and increased sense of well-being.

MER/29 HAS PRODUCED FEW SIDE EFFECTS, NO TOXICITY: Patients have been treated with MER/29 for continuous periods up to 19 months. In no case has there been evidence of serious toxic effects on the function of any vital organ or system. Side effects (nausea, headache, dermatitis) are rare and have usually been associated with dosages greater than those recommended for effective therapy.

MER/29 is compatible with other cardiovascular therapies. It can be used along with measures which control anxiety, hypertension, obesity and other conditions associated with cardiovascular disorders. These include nitroglycerin, PETN, and anticoagulants.

CAUTION: Since long-term MER/29 therapy may be necessary, periodic examinations, including liver function tests, are desirable. Also, since MER/29 inhibits cholesterol biosynthesis, and cholesterol plays an important role in the development of the fetus, the drug is *contraindicated in pregnancy*.

DOSAGE: One 250 mg. capsule daily, before breakfast.

SUPPLIED: Bottles of 30 pearl gray capsules.
Complete bibliography and product information available on request.

MER/29

(triparanol)

Merrell

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Cincinnati, Ohio • Weston, Ontario

Trademark: MER/29®

Can a plumber do a day's work on 1200 calories?

The answer, of course, is "not for long." For example, following diagnosis of diabetes, a 44-year-old plumber (5'8" and weighing 147 lb.) had been put on a 1200-calorie diet to control glycosuria. When referred six months later, he had not been spilling sugar, but had lost 25 pounds and developed progressive fatigability. Orinase, 0.5 Gm./day, was prescribed and his diet was increased to 2800 calories to meet metabolic demands (125 Gm. protein; 300 Gm. carbohydrate; 125 Gm. fat).

Follow-up visits showed this progress:

3 mo. Urine and blood sugar o.k.; weight gain: 28 lb. Can work normally, feels generally well.

6 mo. Weight constant, control constant, no complaints.

12 mo. Same.

18 mo. Same.

24 mo. Same.

Diet-controlled diabetics who are underweight, tire easily, or have increased nutritional needs may merely be "getting by" on dietotherapy alone. These patients – and others who experience transient weakness or listlessness–can often be returned to near-normal activity by giving Orinase together with a more adequate diet. Orinase control of diabetes is notably smooth and stable; patients report a greater sense of well-being, an improved mood and outlook.

Case data courtesy Henry Dolger, M.D.

Indications and effects: The clinical indication for Orinase is stable diabetes mellitus. Its use brings about the lowering of blood sugar; glycosuria diminishes, and such symptoms as pruritus, polyuria, and polyphagia disappear.

Dosage: There is no fixed regimen for initiating Orinase therapy. A simple and effective method is as follows: First day — 6 tablets; second day — 4 tablets; third day — 2 tablets. The daily dose is then adjusted — raised, lowered or maintained at the two-tablet level, whichever is necessary to maintain optimum control.

In patients being converted from insulin, insulin is gradually withdrawn in accordance with the response to Orinase observed over a trial period that may extend to three or four weeks. In candidates for combined Orinase-insulin therapy, an individualized schedule is usually obtainable during a trial course of two or more weeks.

Contraindications and side effects: Orinase is contraindicated in patients having juvenile or growth-onset, unstable or brittle types of diabetes mellitus; history of diabetic coma, fever, severe trauma or gangrene.

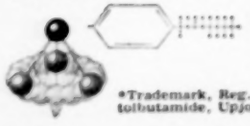
Side effects are mild, transient and limited to approximately 3% of patients. Hypoglycemia and toxic reactions are extremely rare. Hypoglycemia is most likely to occur during the period of transition from insulin to Orinase. Other untoward reactions to Orinase are usually not of a serious nature and consist principally of gastrointestinal disturbances, headache, and variable allergic skin manifestations. The gastrointestinal disturbances (nausea, epigastric fullness, heartburn) and headache appear to be related to the size of the dose, and they frequently disappear when dosage is reduced to maintenance levels or the total daily dose is administered in divided portions after meals. The allergic skin manifestations (pruritus, erythema, and urticarial, morbilliform, or maculopapular eruptions) are transient reactions, which frequently disappear with continued drug administration. However, if the skin reactions persist, Orinase should be discontinued.

Clinical toxicity: Orinase appears to be remarkably free from gross clinical toxicity on the basis of experience accumulated during more than four years of clinical use. Crystalluria or other untoward effects on renal function have not been observed. Long-term studies of hepatic function in humans and experience in over 600,000 diabetics have shown Orinase to be remarkably free of hepatic toxicity. There has been reported only one case of cholestatic jaundice related to Orinase administration, which occurred in a patient with pre-existing liver disease and which rapidly reversed upon discontinuance of the drug.

Each tablet contains:
Tolbutamide ..... 0.5 Gm.
Supplied: In bottles of 50.

Orinase*

An exclusive methyl "governor" prevents hypoglycemia.



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Kalamazoo
Michigan

*Trademark, Reg. U.S. Pat. Off. tolbutamide, Upjohn

Hypertension of 7 years' duration yields to Ser-Ap-Es®

Photo used with patient's permission.

Combination brings blood pressure down after other agents fail—During the past 7 years, Mrs. E. A.'s hypertension gradually advanced in severity. In 1956 and 1957 multiple retinal hemorrhages occurred in the right eye, and vision in this eye deteriorated. Retinopathy advanced to Grade III; EKG showed left ventricular hypertrophy; renal studies showed increasing involvement.

A wide variety of antihypertensive agents (including ganglionic blockers) failed to stabilize blood pressure at satisfactory levels or caused troublesome side effects.

When therapy with Ser-Ap-Es was started, Mrs. A.'s blood pressure (sitting and standing) was 230/120 mm. Hg. With Ser-Ap-Es, blood pressure (sitting and standing) has now been reduced to 190/90, and Mrs. E. A. enjoys a measure of control that had not been achieved with previous agents.

Because it provides 4 actions—central, cardiac, renal, and vascular—in one convenient tablet, Ser-Ap-Es can help you bring more of your hypertensive patients under control.

SUPPLIED: *Tablets* (salmon pink), each containing 0.1 mg. Serpasil, 25 mg. Apresoline hydrochloride, and 15 mg. Esidrix.

For complete information about Ser-Ap-Es (including dosage, cautions, and side effects), see Physicians' Desk Reference or write CIBA, Summit, N. J. 2/2933MR

SERPASIL® (reserpine CIBA)
APRESOLINE® hydrochloride (hydralazine hydrochloride CIBA)
ESIDRIX® (hydrochlorothiazide CIBA)

The actions of Serpasil®, Apresoline® and Esidrix® in a single tablet:

CIBA

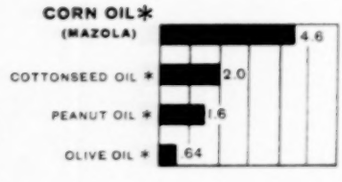
Of all leading brands
of vegetable oils...

Mazola®
liquid corn oil
is best for replacing saturated fats

because Mazola is both unexcelled in polyunsaturates (that reduce serum cholesterol) and lowest in saturates (that raise serum cholesterol).

HERE'S PROOF:

This chart shows that the ratio of polyunsaturates to saturates provided by Mazola Corn Oil is more than twice that of any other leading vegetable oil.



*U. S. Dept. of Agriculture Reports—1959

COMPOSITION OF MAZOLA CORN OIL

Mazola Corn Oil has the following average composition:

Fatty Acids	Grams/ 100 grams	Grams/ fl. oz. (2 tablespoons)
Polyunsaturates	52-58	14-15.7
Monounsaturates	28-36	7.5-9.7
Saturates	10-14	2.8-3.8
Natural Sitosterols	1 (0.9-1.3)	0.14
Natural Tocopherols	about 0.1	0.015
Cholesterol	none	none
Salt (Sodium chloride)	none	none

Calories—125/tablespoon
Iodine value—124 average

CORN OIL CONTINUES TO BE A PREFERRED VEGETABLE OIL IN NUTRITIONAL STUDIES OF HYPERCHOLESTEROLEMIA.

Your patients will find Mazola Corn Oil ideally suited for salad dressings, baking and frying. By instructing them to use Mazola in place of a substantial portion of more saturated types of fat, and to watch their caloric intake, you frequently will be able to lower the serum cholesterol with minimum changes in eating habits.

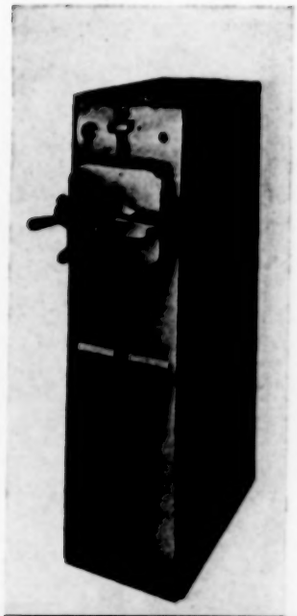
FREE!

Valuable aid for guiding your hypercholesterolemic patient in dietary control: **A Pad of Menu Guides** based on 2,000 calories per day, with which you may readily construct a diet pattern best suited to your patient's needs.

Just write on your letterhead to:
CORN PRODUCTS COMPANY,
10 EAST 56 STREET, NEW YORK 22, N. Y.

MAZOLA CORN OIL IS ALSO AVAILABLE IN CANADA, WESTERN EUROPE, LATIN AMERICA AND MANY OTHER COUNTRIES THROUGHOUT THE WORLD

The 613-R Dynaclave
Low-cost, high-speed autoclave — portable automatic — efficient



The 1022 Aristocrat Autoclave

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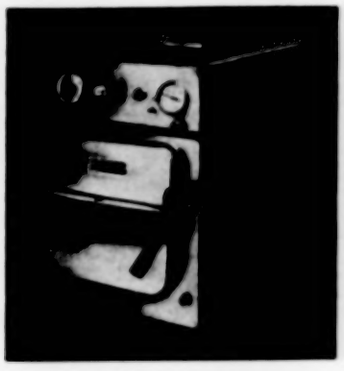
CAN NOW GIVE PATIENTS THE POSITIVE PROTECTION OF PRESSURE STEAM STERILIZATION



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Redesigned to meet the same exacting sterilization standards of the 8816, but at substantially lower cost and with greater capacity.

One of these Amsco Autoclaves can substantially aid your efforts toward improved patient protection against the contaminated needle, or other instruments in your office.

Assurance of the positive protection of pressure steam sterilization is a comfort appreciated most highly by the physician who has faced the problem of cross-contamination. There is an authorized Amsco Dealer near you — ready to advise and serve your requirements for sterilization equipment and adequate techniques. Mailing this coupon with your letterhead will bring full details . . .

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Send Bulletin on Autoclaves 613-R ☐ 8816M ☐ 1022 ☐ and location of nearest Amsco Dealer

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BECAUSE POOR DIABETIC CONTROL INCREASES THE THREAT OF VASCULAR COMPLICATIONS IN DIABETES[1, 2]... DIABINESE FIRST FOR ADEQUATE AND CONTINUOUS ORAL CONTROL

Oral therapy with DIABINESE can help assure more adequate blood-sugar control in many maturity-onset diabetics, including certain patients now poorly controlled by diet alone, some patients on insulin, and many who escape control on previous oral therapy.

Diabinese and diet

In patients with maturity-onset diabetes whose blood sugar remains elevated despite weight and/or caloric control, DIABINESE is frequently effective in doses of 100 to 250 mg. a day. Further, unlike insulin, DIABINESE has not been reported to increase appetite, and residual capacity for endogenous beta cell activity is stimulated. Thus, DIABINESE combined with dietary regulation will often ensure more satisfactory control than "diet alone."

Diabinese and the insulin patient

DIABINESE has proved to be an effective replacement for insulin among maturity-onset patients needing 40 units or less per day. This application of DIABINESE is especially valuable in patients who should not be exposed to the hazards and inconvenience of self-administered injection—those with poor eyesight, the infirm and elderly, and the emotionally disturbed. Transfer from insulin to DIABINESE in proper dosage lessens the risk of hypoglycemia, and may enable certain patients to resume occupations where insulin shock is considered dangerous.

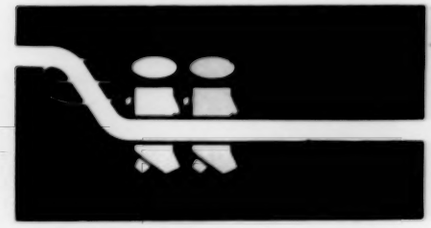
In selected patients in whom insulin requirements have become quite high, combined therapy with DIABINESE sometimes permits reduction of insulin dosage and helps to improve control.[3] Patients with insulin resistance may sometimes be similarly helped by replacement of part of the daily insulin dosage.[4]

Diabinese from the start

Continuous control in suitable candidates for sulfonylurea therapy is more likely to be achieved with DIABINESE. According to the A.M.A. Council on Drugs,[5] observations indicate that "on an equivalent dose and blood level basis, chlorpropamide has a somewhat greater therapeutic effect than has tolbutamide." This therapeutic superiority is reflected in the results of clinical observations like those of Fineberg,[6] who compared the effect of DIABINESE in 50 patients with the effect of tolbutamide in 35 patients. He concluded that "chlorpropamide produced satisfactory control of the diabetes in almost twice as great a percentage (76 versus 43 per cent) of patients than did tolbutamide, and excellent control in more than twice as great a percentage (74 versus 31 per cent)."

1. Johnsson, S.: Diabetes 9:1, 1960. 2. El Mahallawy, M. N., and Sabour, M. S.: J.A.M.A. 173:1783, 1960. 3. Editorial: Brit. M. J. 1:188, 1961. 4. Duncan, L. J. P., and Baird, J. D.: Pharmacol. Rev. 12:91, 1960. 5. A.M.A. Council on Drugs: New and Nonofficial Drugs, 1961, Philadelphia, Lippincott, 1961, p. 657. 6. Fineberg, S. K.: J. Am. Geriat. Soc. 8:441, 1960.

FOR MAXIMUM ASSURANCE OF CONTINUOUS BLOOD-SUGAR CONTROL



Diabinese

the oral antidiabetic most likely to succeed

economical once-a-day dosage

IN BRIEF

DIABINESE, a potent sulfonylurea, provides smooth, long-lasting control of blood sugar permitting economy and simplicity of low, once-a-day dosage. Moreover, DIABINESE often works where other agents have failed to give satisfactory control.

INDICATIONS: Uncomplicated diabetes mellitus of stable, mild or moderately severe nonketotic, maturity-onset type. Certain "brittle" patients may be helped to smoother control with reduced insulin requirements.

ADMINISTRATION AND DOSAGE: Familiarity with criteria for patient selection, continued close medical supervision, and observance by the patient of good dietary and hygienic habits are essential.

Like insulin, DIABINESE dosage must be regulated to individual patient requirements. Average maintenance dosage is 100-500 mg. daily. For most patients the recommended starting dose is 250 mg. given once daily. Geriatric patients should be started on 100-125 mg. daily. A priming dose is not necessary and should not be used; most patients should be maintained on 500 mg. or less daily. Maintenance dosage above 750 mg. should be avoided. Before initiating therapy, consult complete dosage information.

SIDE EFFECTS: In the main, side effects, e.g., hypoglycemia, gastrointestinal intolerance, and neurologic reactions, are related to dosage. They are not encountered frequently on presently recommended low dosage. There have been, however, occasional cases of jaundice and skin eruptions primarily due to drug sensitivity; other side effects which may be idiosyncratic are occasional diarrhea (sometimes sanguineous) and hematologic reactions. Since sensitivity reactions usually occur within the first six weeks of therapy, a time when the patient is under very close supervision, they may be readily detected. Should sensitivity reactions be detected, DIABINESE should be discontinued.

PRECAUTIONS AND CONTRAINDICATIONS: If hypoglycemia is encountered, the patient must be observed and treated continuously as necessary, usually 3-5 days, since DIABINESE is not significantly metabolized and is excreted slowly. DIABINESE as the sole agent is not indicated in juvenile diabetes mellitus and unstable or severely "brittle" diabetes mellitus of the adult type. Contraindicated in patients with hepatic dysfunction and in diabetes complicated by ketosis, acidosis, diabetic coma, fever, severe trauma, gangrene, Raynaud's disease, or severe impairment of renal or thyroid function. DIABINESE may prolong the activity of barbiturates. An effect like that of disulfiram has been noted when patients on DIABINESE drink alcoholic beverages.

SUPPLIED: As 100 mg. and 250 mg. scored chlorpropamide tablets.

More detailed professional information available on request.

Science for the world's well-being®

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after eleven million treatment courses*...

through the years... consistently broad antibacterial action against urinary tract pathogens

"It was interesting to observe that nitrofurantoin [FURADANTIN] showed a consistent in vitro effectiveness against the bacteria tested throughout the four year period, thus revealing negligible development of bacterial resistance, if any, through the years."[1]

*Conservative estimate based on the clinical use of FURADANTIN tablets and Oral Suspension since 1953.

consistently broad antibacterial action

"...was given continuously and safely for as long as three years."[2]

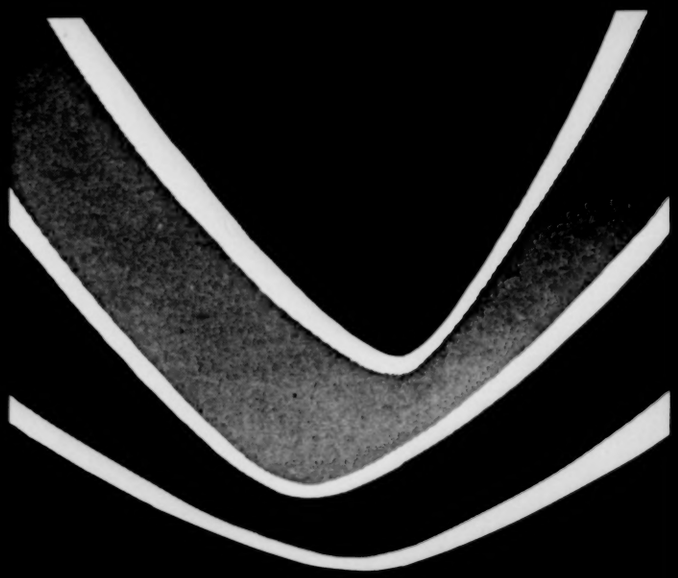
1. Jolliff, C. R., et al.: Antibiot. Chemother. (Wash.) *10*:694, 1960.
2. Lippman, R. W., et al.: J. Urol., Balt. *80*:77, 1958.

Furadantin®

brand of nitrofurantoin

rapid, safe control of infection throughout the urinary system

EATON LABORATORIES, Division of The Norwich Pharmacal Company NORWICH, NEW YORK



TRIMAGILL®*

a new, rational,
convenient
therapy for

WHAT IS TRIMAGILL?

Trimagill is presented as a powder for insufflation and as dry, non-greasy vaginal inserts containing Tartaric Acid, Citric Acid, Boric Acid, Dextrose, Potassium Bitartrate, Potassium Alum, and Adhesives.

TRIMAGILL IS LOGICAL!

Pathogenic micro-organisms that cause vaginal infections are incapable of surviving or propagating in a low pH environment. Trimagill produces and maintains a vaginal pH of 2.0 to 2.5—thus, infecting organisms are destroyed because an unfavorable environment is created.

TRIMAGILL IS EFFECTIVE!

Trimagill's low pH favors the growth of beneficial Döderlein bacilli and helps restore vaginal flora following infections. *Unlike antibiotics Trimagill does not foster monilia overgrowth. Resistant strains cannot develop.*

TRIMAGILL IS PRACTICAL AND CONVENIENT!

Trimagill Powder adheres to the vaginal mucosa for several hours *—eliminates need for vaginal and introital packs or external pads.* Trimagill Powder is easily applied during office visits; Trimagill Vaginal Inserts are recommended for patient use between office visits.

UNINTERRUPTED MEDICATION!

Trimagill treatment may safely be continued during menstruation thus preventing the normal physiological change from an acid to an alkaline pH.

TRIMAGILL IS SAFE!

No untoward reactions have been reported in over 3,000 cases treated to date. The combination of ingredients in Trimagill produces an unusually low pH *with emollient properties that prevent irritation of mucous membranes.*

TRIMAGILL IS PROVED BY CLINICAL EXPERIENCE!

Published papers† representing years of clinical experience in over 3,000 patients demonstrate the effectiveness and safety of Trimagill. Trimagill was used successfully in these cases primarily for acidification of the vaginal tract in treatment of vaginal infections. It was also used and is recommended as a non-absorbable agent following conization of the cervix to help eliminate postoperative sloughing, perineal odor, absorb secretion and maintain an acid pH.

TRIMAGILL IS SUPPLIED:

As *Powder:* 5 oz. Plastic Insufflator Bottles; *As Vaginal Inserts:* Boxes of 24. NOTE: Consult package circular for full details on instructions for use of both Powder and Vaginal Inserts.

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*Patent Applied For.

†Reprints of published papers available on request.

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DAILY DIET
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Meat is one of nature's best sources of complete protein. In addition, meat contributes important B vitamins and significant amounts of essential minerals: iron, copper, phosphorus, magnesium and potassium.

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When the evidence indicates smooth muscle spasm,

good judgment can render it "null and void"
by a ruling in favor of
dependable autonomic sedation

DONNATAL®

TABLETS • CAPSULES • ELIXIR • EXTENTABS

Natural belladonna alkaloids in optimal synergistic ratio, plus phenobarbital

A. H. ROBINS CO., INC., Richmond 20, Virginia

	In each Tablet, Capsule or tsp. (5 cc.) of Elixir	In each Extentab
Hyoscyamine sulfate	0.1037 mg.	0.3111 mg.
Atropine sulfate	0.0194 mg.	0.0582 mg.
Hyoscine hydrobromide	0.0065 mg.	0.0195 mg.
Phenobarbital	(¼ gr.) 16.2 mg.	(¾ gr.) 48.6 mg.

painful skeletal muscle spasm

ROBAXIN®

Robins

INJECTABLE AND TABLETS Methocarbamol Robins U.S. Pat. No. 2770649

Relaxation – obtained *within minutes* with ROBAXIN Injectable.
– maintained *without drowsiness* with ROBAXIN Tablets.

Nine published studies show:

Beneficial results in 90% of cases of skeletal muscle spasm with ROBAXIN.

Clinical responses to ROBAXIN therapy, as reported by investigators:

"*marked*" in 26 out of 33 patients, moderate in 6...[1] "*pronounced*" in 37 out of 58 patients, moderate in 20...[2] "*good*" in 25 out of 38 patients, moderate in 6...[5] "*excellent*" in 14 out of 17 patients, moderate in 2...[6] "*significant*" in 27 out of 30 patients...[7] "*gratifying*" in 55 out of 60 patients...[8] "*effective*" in 32 out of 32 patients...[9] "*marked*" in 27 out of 46 patients, moderate in 6...[4] "*good*" in 57 out of 60 patients, moderate in 3.[10]

ROBAXIN exhibits "great freedom from undesired side reactions,"[3] does not produce "concomitant euphoria or partial anesthesia,"[10] and permits patients to retain concentration and awareness.[8]

For immediate relaxation of acute skeletal muscle spasm:

Robaxin® Injectable — **each ampul containing 1.0 Gm. of methocarbamol in 10 cc. of sterile solution.**

For initiating therapy or maintaining relaxation induced by* ROBAXIN *Injectable:

Robaxin® Tablets — **0.5 Gm. (white, scored) in bottles of 50 and 500.**

Also available: *When pain and spasm require concurrent analgesic and relaxant action:*

Robaxisal® Tablets — **Robaxin with Aspirin**

—and for skeletal muscle relaxation with more comprehensive analgesia:

Robaxisal®— PH — **Robaxin with Phenaphen®**

Literature available to physicians on request.

REFERENCES: 1. Carpenter, E. B.: Southern M.J. 51:627, 1958. **2.** Forsyth, H. F., J.A.M.A. 167:163, 1958. **3.** Hudgins, A. P.: Clin. Med. 6:2321, 1959. **4.** Grisolia, A., and Thomson, J. E. M.: Clin. Orthopaedics 13:299, 1959. **5.** Lewis, W. B.: California Med. 90:26, 1959. **6.** O'Doherty, D. S., and Shields, C. D.: J.A.M.A. 167:160, 1958. **7.** Park, H. W.: J.A.M.A. 167:168, 1958. **8.** Plumb, C. S.: Journal-Lancet 78:531, 1958. **9.** Poppen, J. L., and Flanagan, M. E.: J.A.M.A. 171:298, 1959. **10.** Schaubel, H. J.: Orthopedics 1:274, 1959.

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Menopausal distress: a syndrome involving all three levels of the autonomic nervous system

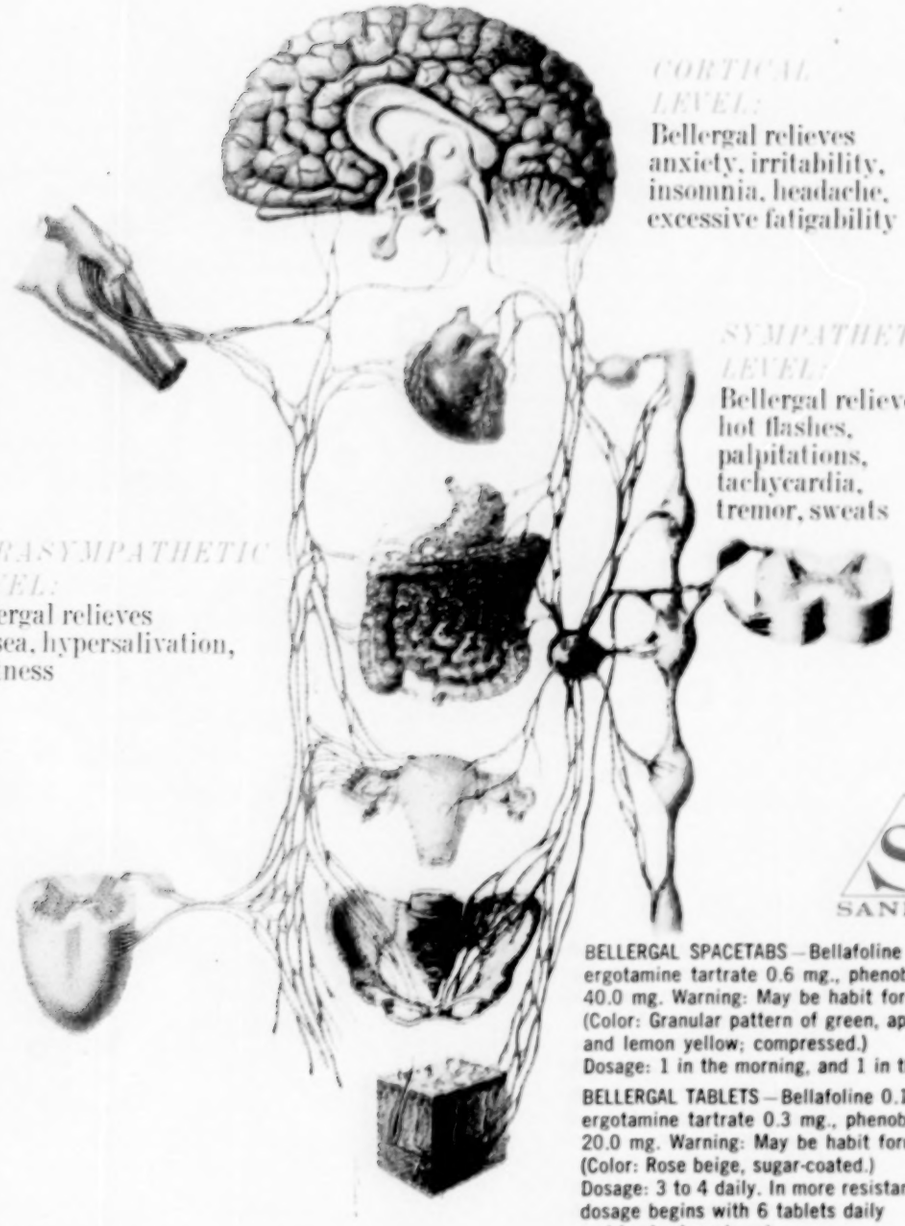
for functional disorders of the menopause

Bellergal®

SPACETABS®

stabilizes the entire autonomic nervous system

(without disturbing endocrine balance)



CORTICAL LEVEL:
Bellergal relieves anxiety, irritability, insomnia, headache, excessive fatigability

SYMPATHETIC LEVEL:
Bellergal relieves hot flashes, palpitations, tachycardia, tremor, sweats

PARASYMPATHETIC LEVEL:
Bellergal relieves nausea, hypersalivation, faintness

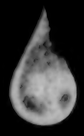
SANDOZ

BELLERGAL SPACETABS—Bellafoline 0.2 mg., ergotamine tartrate 0.6 mg., phenobarbital 40.0 mg. Warning: May be habit forming. (Color: Granular pattern of green, apricot and lemon yellow; compressed.)
Dosage: 1 in the morning, and 1 in the evening.

BELLERGAL TABLETS—Bellafoline 0.1 mg., ergotamine tartrate 0.3 mg., phenobarbital 20.0 mg. Warning: May be habit forming. (Color: Rose beige, sugar-coated.)
Dosage: 3 to 4 daily. In more resistant cases, dosage begins with 6 tablets daily and is slowly reduced.

aspirin buffered with the most widely-prescribed antacid...

Aspirin
300 mg
5 gr



MAALOX
150 mg

ASCRIPTIN

in long-term administration, as in Arthritis,
when aspirin combined with an antacid is desired:

Specify **Ascriptin**® RORER

the aspirin buffered with the best

To prevent or minimize gastric distress which often accompanies prolonged or high level administration of acetylsalicylic acid, ASCRIPTIN provides aspirin in combination with MAALOX®, the preferred professional antacid. The recognized superiority of MAALOX makes ASCRIPTIN a superior aspirin-antacid, with the virtues of buffered aspirin and with the added distinction of being promoted professionally only.

Indicated wherever salicylates are useful, ASCRIPTIN is particularly suited to the long-term requirements of your arthritic patients.

Supplied: Bottles of 100 and 500 tablets. For severe pain – Capsules ASCRIPTIN with Codeine (codeine phosphate 15 mg.), bottles of 50.

WILLIAM H. RORER, INC. PHILADELPHIA, PENNSYLVANIA

A "NEST EGG" HELPS PROVIDE A SECURE FUTURE...

ELDEC®
KAPSEALS®

HELP PROVIDE A HEALTHY ONE

by supplying a dependable source of vitamins, minerals, hormones, digestive enzymes, and amino acids.

Each ELDEC Kapseal contains vitamins—1667 units *A*, 0.67 mg. B_1 mononitrate, 0.67 mg. B_2, 0.5 mg. pyridoxine hydrochloride, 0.033 N.F. Unit (Oral) B_{12} with intrinsic factor concentrate, 0.1 mg. folic acid, 33.3 mg. C, 16.7 mg. nicotinamide, 10 mg. *dl*-panthenol, 6.67 mg. choline bitartrate; minerals—16.7 mg. ferrous sulfate (exsiccated), 0.05 mg. iodine (as potassium iodide), 66.7 mg. calcium carbonate; digestive enzymes—20 mg. Taka-Diastase® (Aspergillus oryzae enzymes), 133.3 mg. pancreatin; amino acids—66.7 mg. *l*-lysine monohydrochloride, 16.7 mg. *dl*-methionine; gonadal hormones—1.67 mg. methyltestosterone, 0.167 mg. Theelin.

Dosage: One Kapseal three times daily before meals. Female patients should follow each 21-day course with a 7-day rest interval.

Precautions: Contraindicated in patients wherein estrogen or androgen therapy should not be used, as in carcinoma of the breast, genital tract, or prostate, and in patients with a familial tendency to these types of malignancy; give cautiously to females who tend to develop excessive hair growth or other signs of masculinization.

Packaging: ELDEC Kapseals are available in bottles of 100.

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"Dr. B" on NBC-TV
TUESDAY NIGHT, JUNE 27, 1961
A portrait of the family physician and his special contributions to the health of the nation

HYPERTENSIVE INVALIDISM:

headache
dizziness
palpitations
tachycardia
anginal pain
anxiety
organic changes
edema
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THE DIFFERENCE .

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DIURIL® CHLOROTHIAZIDE WITH RESERPINE

- the first "wide range" antihypertensive
- effective by itself in a majority of patients with mild or moderate hypertension, and even in many with severe hypertension
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MORE NORMAL LIFE:

hypertensive symptoms are usually relieved

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DIUPRES-250

250 mg. DIURIL chlorothiazide, 0.125 mg. reserpine per tablet. One tablet one to four times a day.*

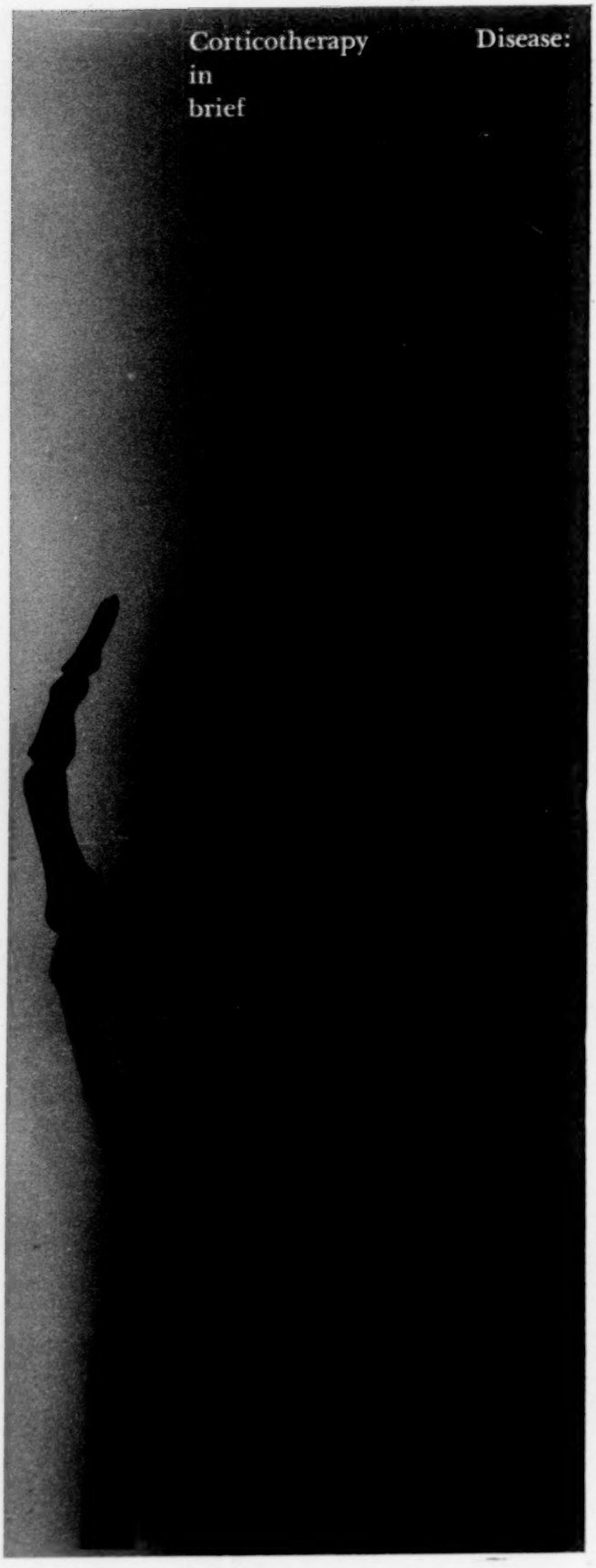
DIUPRES-500

500 mg. DIURIL chlorothiazide, 0.125 mg. reserpine per tablet. One tablet one to three times a day.*

*It is essential to reduce the dosage of other antihypertensive agents, particularly the ganglion-blockers, by at least 50 per cent immediately upon addition of these agents or of Diupres Tablets to the regimen.

Before prescribing or administering DIUPRES, the physician should consult the detailed information on use accompanying the package or available on request.

MSD **MERCK SHARP & DOHME**
DIVISION OF MERCK & CO., Inc., WEST POINT, PA.



Rheumatoid arthritis

Use of oral Medrol:

In severe or moderately severe cases, initial dosage of Medrol tablets is 8 to 16 mg. daily; maintenance dosage ranges from 4 to 12 mg. daily, adjusted stepwise every 5 to 10 days in accordance with response. In children, and also in adults with moderate disease, both initial and maintenance dosage is Medrol 4 to 8 mg. daily.

"It [methylprednisolone] is potent and displays a slightly improved 'safety' record, showing a reduced frequency of disturbing side-effects as compared with the other steroids."

—Neustadt, D. H.: J.A.M.A. *170*:1253 (July 11) 1959.

Medrol*

Upjohn

75th year

Indications and effects

Medrol benefits (anti-inflammatory, antiallergic, antirheumatic, antileukemic, antihemolytic) have been demonstrated in acute rheumatic carditis, rheumatoid arthritis, asthma, hay fever and allergic disorders, dermatoses, blood dyscrasias, and ocular inflammatory disease involving the posterior segment.

Precautions and contraindications

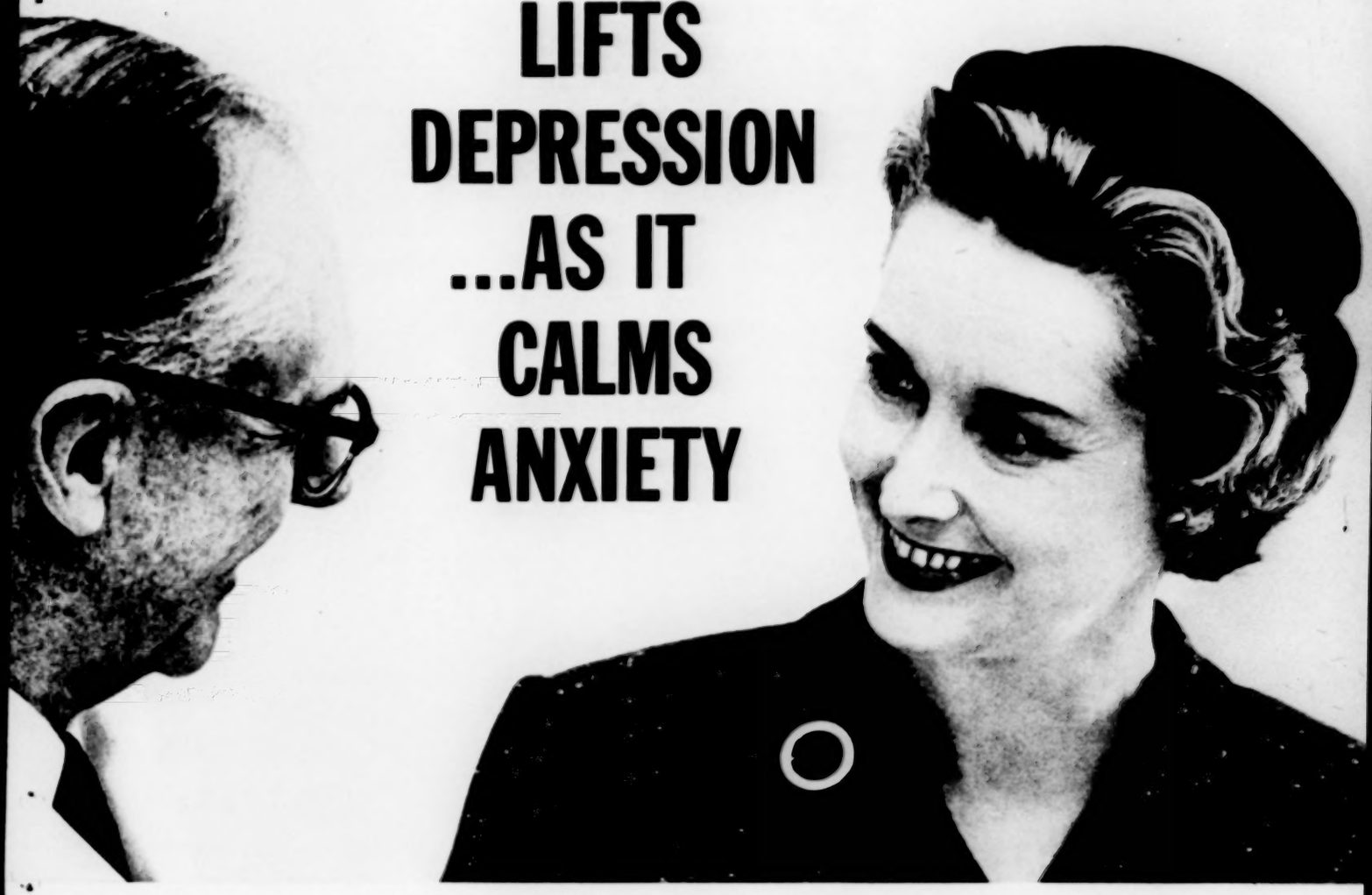
Because of Medrol's high therapeutic ratio, patients usually experience dramatic relief *without* developing such possible steroid side effects as gastrointestinal intolerance, weight gain or weight loss, edema, hypertension, acne, or emotional imbalance.

As in all corticotherapy, however, there are certain cautions to be observed. The presence of diabetes, osteoporosis, chronic psychotic reactions, predisposition to thrombophlebitis, hypertension, congestive heart failure, renal insufficiency, or active tuberculosis necessitates careful control in the use of steroids. Like all corticosteroids, Medrol is contraindicated in patients with arrested tuberculosis, peptic ulcer, acute psychoses, Cushing's syndrome, herpes simplex keratitis, vaccinia, or varicella.

Each tablet contains: Medrol (*methylprednisolone*) . . . . . 2, 4, or 16 mg.
Medrol is supplied as 2 mg. tablets in bottles of 30 and 100; as 4 mg. tablets in bottles of 30, 100 and 500; and as 16 mg. tablets in bottles of 50.

*Trademark, Reg. U. S. Pat. Off.
The Upjohn Company, Kalamazoo, Michigan

LIFTS DEPRESSION ...AS IT CALMS ANXIETY



"I feel like my old self again!" Thanks to your balanced Deprol therapy, normal drive and interest have replaced her emotional fatigue.

Brightens up the mood, brings down tension

Balanced action—avoids "seesaw" effects of energizers and amphetamines.

Acts rapidly—you see improvement in a few days.

Acts safely—no danger of liver or blood damage.

Dosage: Usual starting dose is 1 tablet q.i.d. When necessary, this dose may be gradually increased up to 3 tablets q.i.d.

Composition: 1 mg. 2-diethylaminoethyl benzilate hydrochloride (benactyzine HCl) and 400 mg. meprobamate.

Supplied: Bottles of 50 light-pink, scored tablets.

WALLACE LABORATORIES / *Cranbury, N. J.*

CD-4690

▲Deprol▲®

Mail this coupon for clinical supply of Deprol

Dept. D-11
Professional Services Dept.
Wallace Laboratories
Cranbury, N. J.

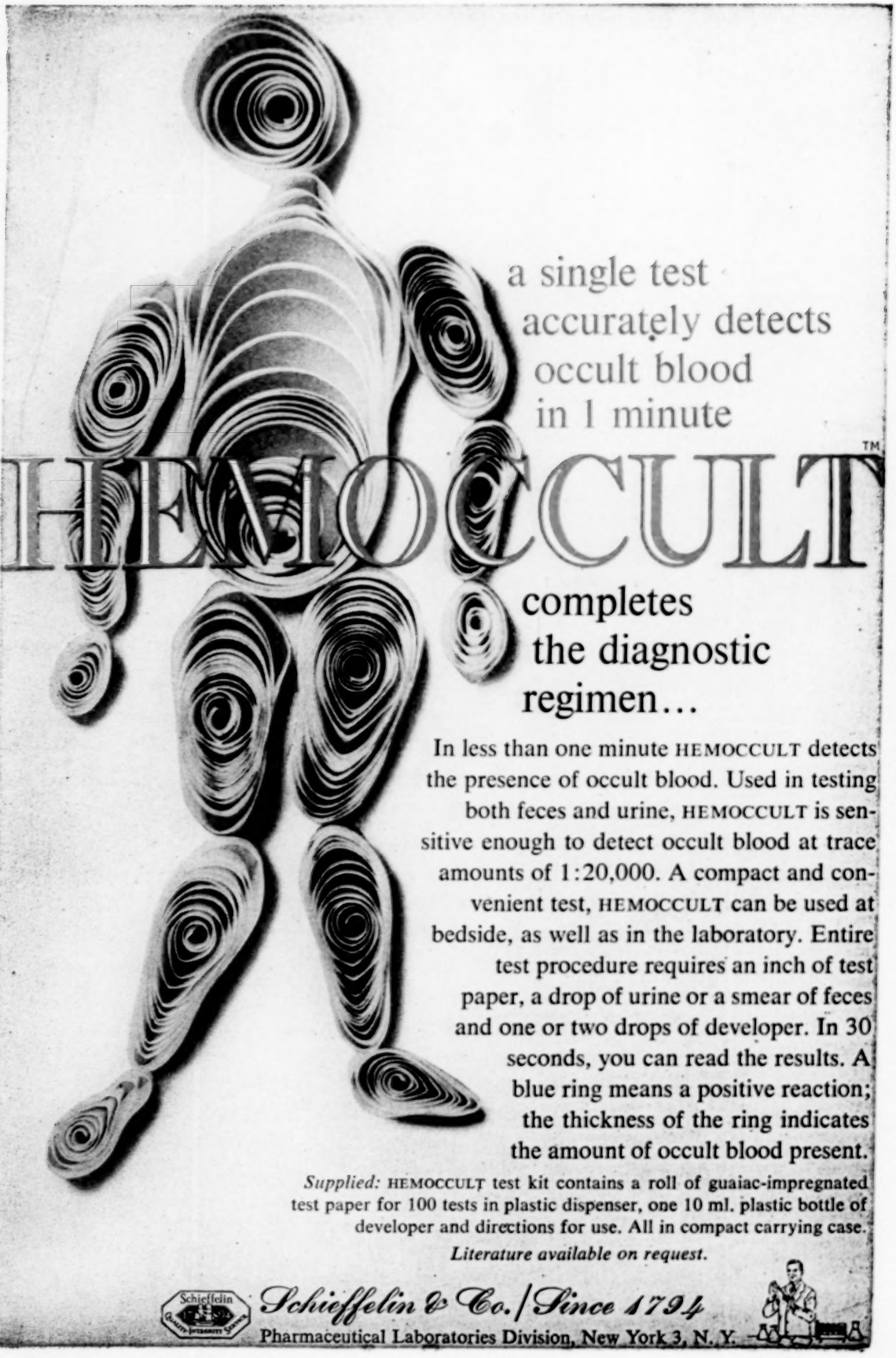
Gentlemen: *Please send me a clinical supply of Deprol for the treatment of depression.*

Dr. .....

Street .....

City ..... Zone ..... State .....

Type of practice .....



a single test
accurately detects
occult blood
in 1 minute

HEMOCCULT™

completes the diagnostic regimen...

In less than one minute HEMOCCULT detects the presence of occult blood. Used in testing both feces and urine, HEMOCCULT is sensitive enough to detect occult blood at trace amounts of 1:20,000. A compact and convenient test, HEMOCCULT can be used at bedside, as well as in the laboratory. Entire test procedure requires an inch of test paper, a drop of urine or a smear of feces and one or two drops of developer. In 30 seconds, you can read the results. A blue ring means a positive reaction; the thickness of the ring indicates the amount of occult blood present.

Supplied: HEMOCCULT test kit contains a roll of guaiac-impregnated test paper for 100 tests in plastic dispenser, one 10 ml. plastic bottle of developer and directions for use. All in compact carrying case.

Literature available on request.

Schieffelin

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a pair of cardiac patients:

both are free of pain—but only one is on

DILAUDID®

(Dihydromorphinone HCl)

swift, sure analgesia normally unmarred by nausea and vomiting

DILAUDID provides unexcelled analgesia in acute cardiovascular conditions. Onset of relief from pain is almost immediate. The high therapeutic ratio of DILAUDID is commonly reflected by lack of nausea and vomiting—and marked freedom from other side-effects such as dizziness and somnolence.

▲ **by mouth** ▲ **by needle** ▲ **by rectum**

2 mg., 3 mg., and 4 mg.

May be habit forming—usual precautions should be observed as with other opiate analgesics.

KNOLL PHARMACEUTICAL COMPANY • ORANGE, NEW JERSEY

IN PEPTIC ULCER AND HYPERACIDITY *with associated* TENSION and NERVOUSNESS

NACTISOL

suppresses gastric acid secretion at the parietal cell level

decreases gastrointestinal hypermotility

relieves nervousness and tension

NACTISOL combines:

NACTON® 4 mg. (poldine methylsulfate†) — new inhibitor of gastric acid secretion and hypermotility "... reduces the total output of gastric HCl by about 60%"[1]

plus

BUTISOL SODIUM® 15 mg. (butabarbital sodium) — "daytime sedative" with highest therapeutic index[2] (highly effective, minimal side effects)

smooth, predictable sedation of *6 hours' duration*

• Side effects with NACTISOL therapy have been minimal.[3-5]

NACTISOL*...in scored, yellow tablets—bottles of 100

Typical gastric secretory gland.

a —chief cell (pepsin-producing).

b —parietal cell (acid-producing).

NACTISOL INHIBITS GASTRIC ACID SECRETION AT THE PARIETAL CELL LEVEL

References

1. Douthwaite, A. H.: The Development of the Treatment of Duodenal Ulcer, Proc. Roy. Soc. Med. *51*:1063-1068 (December) 1958.
2. Batterman, R. C., Grossman, A. J., Leifer, P., and Mouratoff, G. J.: Clinical Re-evaluation of Daytime Sedatives, Postgrad. Med. *26*:502-509 (October) 1959.
3. Steigmann, F.: Clinical Report to McNeil Laboratories.
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5. Rider, J. A.: Clinical Report to McNeil Laboratories.

McNEIL McNEIL LABORATORIES, INC., Fort Washington, Pa.

*Trademark
†U. S. Patent

5-year study[1] with COUMADIN demonstrates: long-term anticoagulation in office management of outpatients is practical and effective

A 5-year study[1] of long-term anticoagulation with COUMADIN (warfarin sodium) in office practice patients has demonstrated that such treatment reduces the probability of further infarctions in the postinfarct patient and is effective in preventing a first infarction in patients with angina.

An earlier report[2] noted that long-term anticoagulant therapy with warfarin sodium can be carried out, along with the necessary prothrombin time determinations, as part of general office practice.

"The most significant advantage is the great ease in maintaining patients in a therapeutic range. It has been rewarding to find, month after month, patients varying no more than three or four seconds in their prothrombin times on their established dosage of Warfarin sodium [COUMADIN]."[1]

COUMADIN®

FOR ORAL, INTRAVENOUS OR INTRAMUSCULAR USE

the original and only warfarin responsible for establishing this drug as closely approaching the ideal anticoagulant[3,4] and as "the best anticoagulant [illegible] today."[5] Over 179,000,000 doses [illegible]

the proven anticoagulant for long-term mainte[illegible]

Full range of oral and parenteral dosage forms—COUMADIN® (warfarin sodium) is available as: Scored tablets—2 mg., lavender; 5 mg., peach; 7½ mg., yellow; 10 mg., white; 25 mg., red. Single Injection Units—one vial, 50 mg., and one 2 cc. ampul Water for Injection; one vial, 75 mg., and one 3 cc. ampul Water for Injection.

Average Dose: initial, 40-60 mg. For elderly [illegible] tated patients, 20-30 mg. Maintenance, [illegible] as indicated by prothrombin time determinations [illegible]

1. Nero, J. J.: M. Times, May, 1959. 2. Nero, J. J.: [illegible] 1958. 3. Baer, S., et al.: J.A.M.A. 167:704, June 7, 1958. [illegible] once-a-Month, Chicago, Yr. Bk. Pub., Mar., 1958, p. [illegible] Postgrad. Med. 24:116, Aug., 1958.

*Manufactured under license from the Wisconsin [illegible]

Complete Information and Reprints on Request Endo ENDO LABORATORIES [illegible]

an antibiotic capsule with an added measure of protection

AGAINST RELAPSE — **up to 6 days' activity with 4 days' dosage**

AGAINST "PROBLEM" PATHOGENS — **uniformly sustained peak activity**

AGAINST SECONDARY INFECTION — **full antibiotic response**

DISTINCTIVE, DRY-FILLED, DUOTONE RED CAPSULES — **150 mg., bottles of 16 and 100. Dosage: 1 capsule (150 mg.) four times daily.** Precautions: As with other antibiotics, DECLOMYCIN may occasionally give rise to glossitis, stomatitis, proctitis, nausea, diarrhea, vaginitis or dermatitis. A photodynamic reaction to sunlight has been observed in a few patients on DECLOMYCIN. Although reversible by discontinuing therapy, patients should avoid exposure to intense sunlight. If adverse reaction or idiosyncrasy occurs, discontinue medication. Overgrowth of nonsusceptible organisms is a possibility with DECLOMYCIN, as with other antibiotics. The patient should be kept under constant observation.

Request complete information on indications, dosage, precautions and contraindications from your Lederle representative, or write to Medical Advisory Department.

DECLOMYCIN®

DEMETHYLCHLORTETRACYCLINE LEDERLE

LEDERLE LABORATORIES, a Division of AMERICAN CYANAMID COMPANY, Pearl River, N. Y.

designed
with a
specific
aim

BENYLIN EXPECTORANT

specifically designed to help control cough

Just as a medical instrument is engineered for maximum efficiency in performing its specific function, BENYLIN® EXPECTORANT is formulated to provide effective relief of cough associated with colds or allergy.

This outstanding antitussive action of BENYLIN EXPECTORANT is attributed to a carefully selected combination of therapeutic agents. Benadryl,® a potent antihistaminic-antispasmodic, reduces bronchial spasm, quiets the cough reflex, and lessens nasal stuffiness, sneezing, lacrimation, itching, and other allergic manifestations. Concurrent respiratory congestion is relieved by expectorant agents that efficiently break down tenacious mucosal secretions. In addition, a demulcent action soothes irritated throat membranes. 50461

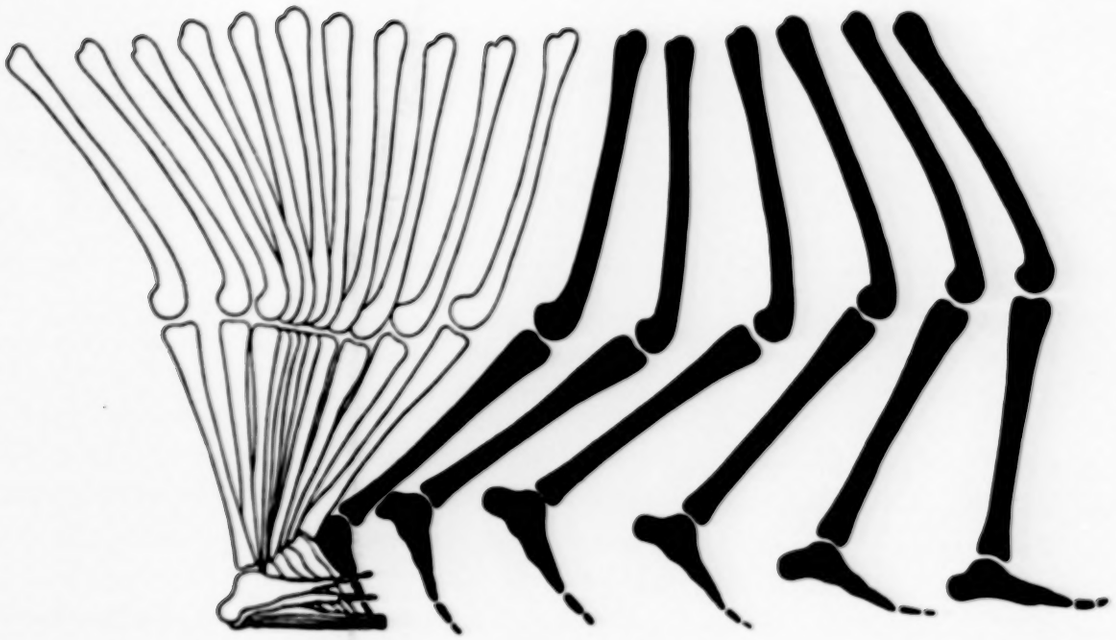
BENYLIN EXPECTORANT is a pleasant-tasting, raspberry-flavored syrup... completely acceptable to patients of all ages.

supplied: BENYLIN EXPECTORANT is available in 16-ounce and 1-gallon bottles.

Each fluidounce contains: 80 mg. Benadryl hydrochloride (diphenhydramine hydrochloride, Parke-Davis); 12 gr. ammonium chloride; 5 gr. sodium citrate; 2 gr. chloroform; 1/10 gr. menthol; and 5% alcohol. *Indications:* Relief of coughs due to colds, and other symptoms associated with colds, and coughs of allergic origin. *Dosage:* Adults—1 to 2 teaspoonfuls every three to four hours. Children—½ to 1 teaspoonful every four hours. *Precautions:* Products containing Benadryl should be used cautiously with hypnotics or other sedatives; if atropine-like effects are undesirable; or if the patient engages in activities requiring alertness or rapid, accurate response (such as driving).

PARKE-DAVIS

PARKE, DAVIS & COMPANY, Detroit 32, Michigan



Depo-Medrol was administered intra-articularly to 118 patients (250 injections) for disorders including rheumatoid arthritis, osteoarthritis, epicondylitis, and tendinitis.

Relief of pain and swelling was marked or complete in 104 of the 118 (88.1%); duration of response to a single injection was more than three weeks in 89 patients (75.4%) and more than six weeks in 39 of these.[1] "Post-injection flare-up was practically non-existent."[1]

relief within hours... lasting for weeks

Depo-Medrol* intra-articularly

Indications and dosages

Intra-articular, intrabursal and intratendinous injections of Depo-Medrol are useful for sustained anti-inflammatory effect and symptomatic relief in rheumatoid arthritis, osteoarthritis, bursitis, tendinitis, epicondylitis and other rheumatic disorders.

Intra-articular dosage depends on the size of the joint and the severity of the condition. Injections may be repeated, if necessary, at intervals of one to five weeks. A suggested dosage guide: Large joint, 20 to 80 mg.; medium joint, 10 to 40 mg.; small joint, 4 to 10 mg.

For administration directly into bursae, dosage may be 4 to 30 mg. (repeat injections are usually not needed).

For injection into the tendon sheath, 4 to 30 mg. is a usual range (in recurrent or chronic conditions, repeat injections may be needed).

Precautions

Depo-Medrol for local effect is contraindicated in the presence of acute infectious conditions. Infrequently, atrophic changes in the dermis may form shallow depressions in the skin at the injection site, but these usually disappear in a few months.

Depo-Medrol 40 mg. per cc.
Each cc. contains:

Medrol (methylprednisolone) acetate	40 mg.
Polyethylene glycol 4000	29 mg.
Sodium chloride	8.7 mg.
Myristyl-gamma-picolinium chloride	0.19 mg.
Water for injection	q.s.

Supplied: 1 cc. and 5 cc. vials

20 mg. per cc.
Each cc. contains:

Medrol (methylprednisolone) acetate	20 mg.
Polyethylene glycol 4000	29.6 mg.
Sodium chloride	8.9 mg.
Myristyl-gamma-picolinium chloride	0.19 mg.
Water for injection	q.s.

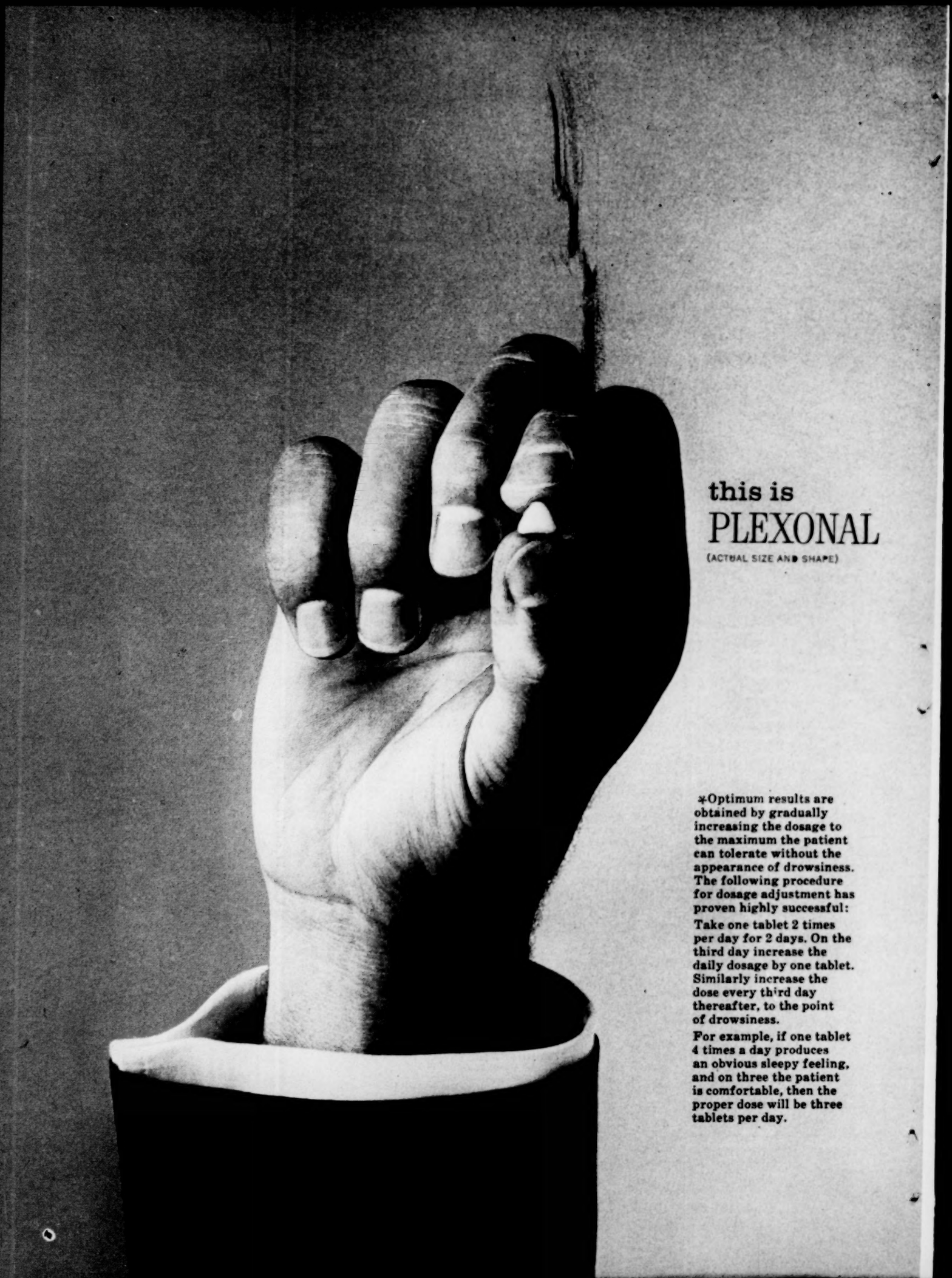
Supplied: 5 cc. vials

1. Norcross, B. M., and Winter, J. A.: Methylprednisolone acetate: a single preparation suitable for both intra-articular and systemic use, New York J. Med. *61*:552 (Feb. 15) 1961.

*Trademark, Reg. U. S. Pat. Off.
methylprednisolone acetate, Upjohn

The Upjohn Company, Kalamazoo, Michigan

Upjohn



this is
PLEXONAL
(ACTUAL SIZE AND SHAPE)

*Optimum results are obtained by gradually increasing the dosage to the maximum the patient can tolerate without the appearance of drowsiness. The following procedure for dosage adjustment has proven highly successful:

Take one tablet 2 times per day for 2 days. On the third day increase the daily dosage by one tablet. Similarly increase the dose every third day thereafter, to the point of drowsiness.

For example, if one tablet 4 times a day produces an obvious sleepy feeling, and on three the patient is comfortable, then the proper dose will be three tablets per day.

a superior daytime relaxing agent

(NOT A TRANQUILIZER)

PLEXONAL®

Comparative clinical studies show that PLEXONAL is superior to meprobamate or barbiturates for daytime relaxation[1,2]

"Plexonal was preferred (superior therapeutic effect) by 73.7 per cent of the patients, whereas 11.1 per cent preferred meprobamate, a ratio of 6.6 to 1. . . . 30.5 per cent noted adverse reactions to meprobamate as compared to 7 per cent in respect to Plexonal. . . . Plexonal gave better results than did any of the sedative or relaxing agents that have been available during our experience covering the previous 15 years."[1]

In 26 older age cardiac patients, "A comparison of Plexonal with the therapy previously employed showed that 17 did better on Plexonal than on meprobamate, 6 did better on meprobamate than on Plexonal and 3 responded the same to both."[2]

Indications: Anxiety, tension, apprehension, nervousness, irritability, restlessness, hyperexcitability.

Extremely well tolerated by geriatric patients who need mild sedation, as well as by depressed patients.

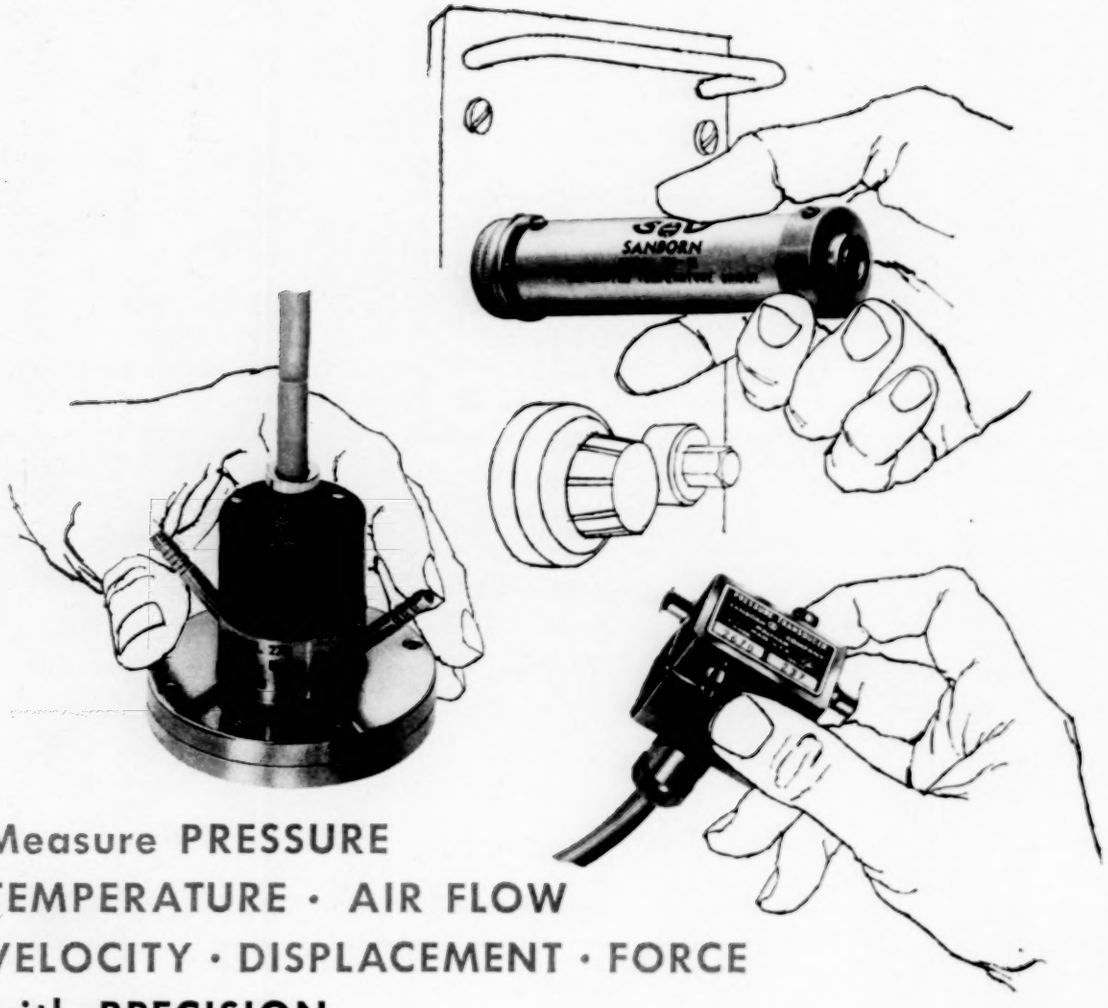
Dosage: One tablet 3 or 4 times a day is adequate for most patients. However, some require up to six tablets per day, whereas others respond adequately to as little as 1 tablet per day.*

Composition: Each tablet contains sodium diethylbarbiturate 45 mg., sodium phenylethylbarbiturate 15 mg., sodium isobutylallylbarbiturate 25 mg., scopolamine hydrobromide 0.08 mg., dihydroergotamine methanesulfonate 0.16 mg.

1. Scheifley, C. H.: Proc. Staff Meet. Mayo Clin. 34:408 (Aug. 19) 1959.
2. Davanloo, H.: Am. J. of Psychiat. 117:740 (Feb.) 1961.

ORIGINAL RESEARCH SERVING THE PHYSICIAN

SANDOZ



Measure PRESSURE TEMPERATURE · AIR FLOW VELOCITY · DISPLACEMENT · FORCE with PRECISION SANBORN TRANSDUCERS

You can meet a wide variety of requirements from this broad selection of accurate, compact Sanborn Transducers. Series 267 and 268 Physiological Pressure Transducers are designed for either differential or single-ended measurements in such applications as cardiac catheterization and studies of circulatory, respiratory, esophogeal, spinal or gastric pressures. Two basic sensitivities are available: 1.0 or 0.1 mm Hg produces 1 cm. chart deflection. Model 270 Bi-Directional Differential Gas Pressure Transducer permits measurements of small pressure changes (1 cm/0.5 mm H_2O) over a wide range with excellent linearity and low drift. Pneumotach heads for respiratory air flow measurements are available for use with the 270. Excellent stability and temperature compensation also make the Model 270 particularly suitable for plethysmography applications.

Model 760-53 Calibrated Temperature Bridge and a variety of interchangeable thermistor probes are available for use with any Sanborn Carrier Preamplifier for accurate recording or monitoring of physiological temperatures with full scale sensitivity of 1°C and 2.5°C.

In addition, Sanborn offers pneumograph and pulse wave attachments, heart sound microphones, linear velocity and displacement transducers, and transducers for force measurements in myographic studies. For complete information contact your nearest Sanborn Branch Office or Service Agency – or write Manager, Research Instrument Sales, Medical Division.

MEDICAL DIVISION

SANBORN COMPANY

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VITAMINS ARE THERAPY

STRESSCAPS®

STRESS FORMULA VITAMINS LEDERLE

IN SEVERE INFECTION

Therapeutic B and C vitamins rapidly restore essential nutrients lost during the acute phase, and help mobilize body defenses during convalescence. STRESSCAPS helps condition the body to respond to primary therapy.

Packaged (30 and 100) in decorative "reminder" jar.

Each capsule contains:

Thiamine Mononitrate (B_1)	10 mg.
Riboflavin (B_2)	10 mg.
Niacinamide	100 mg.
Ascorbic Acid (C)	300 mg.
Pyridoxine HCl (B_6)	2 mg.
Vitamin B_{12}	4 mcgm.
Calcium Pantothenate	20 mg.

Average dose: 1 to 2 capsules daily.

Request complete information on indications, dosage, precautions and contraindications from your Lederle representative, or write to Medical Advisory Department.

LEDERLE LABORATORIES, A Division of AMERICAN CYANAMID COMPANY, Pearl River, New York

Butazolidin

roved by [illegible] of experience

Notable Success with VISTARIL...

in prepartum tension and anxiety		allays anxiety without impairing ability to cooperate during labor and delivery[1]	reduces narcotic requirements and incidence of narcotic-induced respiratory depression, helps control emesis[1,4]
in the cardiac or the hypertensive patient		allays anxiety without adverse influence on blood pressure[2]	helps correct certain functional arrhythmias, does not increase gastric secretion[2]
in problem drinkers		allays anxiety—makes patient more manageable[3]	produces no significant depression of blood pressure, pulse rate, or respiration. No liver involvement reported
in preoperative tension and anxiety		allays anxiety without depression of vital functions[4]	reduces incidence of narcotic-induced respiratory depression and hypotension, relaxes skeletal muscle, smooths recovery and helps control emesis[4]
in pediatrics		allays tension in agitated, hyperkinetic patients	avoids danger of liver damage or other untoward reactions

References: 1. Benson, C., and Benson, R. C.: Scientific Exhibit, Illinois Acad. Gen. Practice, Sept., 1960. 2. Salmons, J. A.: Dis. Chest 38:105, 1960. 3. Major, R. A.: GP 21:104, 1960. 4. Grady, R. W., and Rich, A. L.: Scientific Exhibit, Am. Soc. Anesth., New York, Oct. 4-7, 1960.

for successful
tranquilization—

Vistaril

ORAL/HYDROXYZINE PAMOATE
PARENTERAL/HYDROXYZINE HYDROCHLORIDE

effectively allays anxiety

no reported incidence of liver damage, respiratory depression or addiction

exerts helpful antiemetic, antisecretory, antipruritic effects

Science for the world's well-being™

Pfizer

PFIZER LABORATORIES
Division, Chas. Pfizer & Co., Inc.
Brooklyn 6, New York

IN BRIEF

Vistaril is hydroxyzine pamoate. The hydrochloride salt of hydroxyzine is used in the parenteral solution.

Vistaril acts rapidly in the symptomatic treatment of a variety of neuroses and other emotional disturbances manifested by anxiety, apprehension or fear—whether occurring alone or complicating a physical illness. Used preoperatively and prepartum, Vistaril controls anxiety and fear, permits a substantial reduction in the amount of meperidine or other narcotic required for satisfactory analgesia, and helps prevent emesis. Vistaril's calming effect usually does not impair discrimination, and is accompanied by direct and secondary muscle relaxation. No toxicity has been reported with Vistaril, and it has a remarkable record of freedom from reactions.

INDICATIONS: Vistaril is clinically effective in anxiety and tension states, senility, anxiety associated with various disease states, alcoholism, pre- and postpartum and pre- and postoperative tension and emesis, certain functional arrhythmias, and pediatric behavior problems.

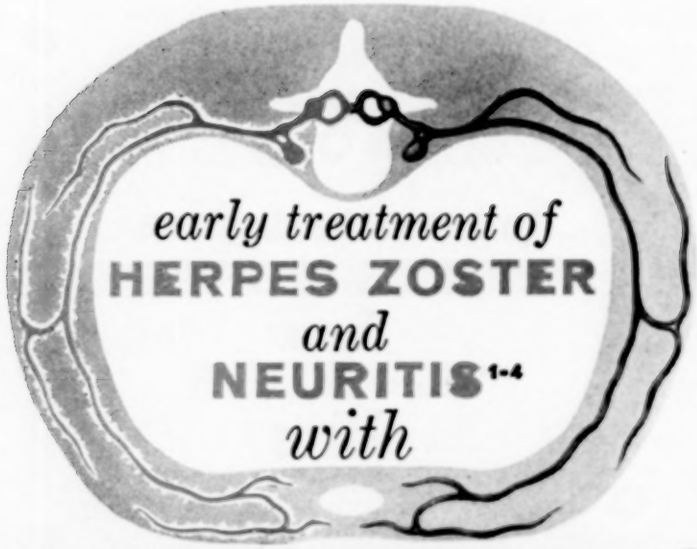
ADMINISTRATION AND DOSAGE: Dosage varies with the state and response of each patient, rather than with weight and should be individualized by the physician for optimum results. *Recommended oral dosage:* In anxiety and tension states, senility, alcoholism, pre- and postoperative and pre- and postpartum tension and emesis: up to 400 mg. daily in divided doses. In anxiety associated with asthma, neurodermatoses, menopausal syndrome, digestive disorders, functional or essential hypertension, tension headaches: 50 mg. q.i.d. initially—adjust according to response. In cardiac arrhythmias: initial—25 mg. q. 6 h. until arrhythmia disappears; maintenance or prophylactic—50-75 mg. daily in divided doses. In pediatric behavior problems under 6 years: 50 mg. daily in divided doses. Six and over: 50-100 mg. daily in divided doses. *Recommended parenteral dosage:* In preoperative, obstetrical, and more emergent situations in other indications: 25-100 mg. I.M. or I.V. q. 4 h., p.r.n. In cardiac arrhythmias: 50-100 mg. I.M. stat, and q. 4-6 h., p.r.n.; maintain with 25 mg. b.i.d. or t.i.d.

SIDE EFFECTS: Drowsiness may occur in some patients; if so, it is usually transitory, disappearing within a few days of continued therapy or upon reduction of dosage. Dryness of mouth may be encountered at higher doses.

PRECAUTIONS: The potentiating action of hydroxyzine should be taken into account when the drug is used in conjunction with central nervous system depressants. Do not exceed 1 cc. per minute I.V. Do not give over 100 mg. per dose I.V. Parenteral therapy is usually for 24-48 hours, except when, in the judgement of the physician, longer-term therapy by this route is desirable.

SUPPLIED: VISTARIL *Capsules* (hydroxyzine pamoate)—25, 50, and 100 mg. VISTARIL *Oral Suspension* (hydroxyzine pamoate)—25 mg. per 5 cc. teaspoonful. VISTARIL *Parenteral Solution* (hydroxyzine hydrochloride)—10 cc. vials, 25 mg. per cc.; 2 cc. ampules, 50 mg. per cc.

More detailed professional information available on request.



PROTAMIDE®

provides rapid relief

Relief of inflammatory radicular pain, including herpes zoster, is prompt when Protamide is administered early[1-4] in the course of the disease. More important, recovery usually follows in three to six days, with prompt response even in ophthalmic herpes zoster.[5]

Published studies suggest that Protamide acts as a direct suppressant of neuritis due to acute inflammation of the nerve root. In such disorders, the response to early treatment with Protamide is sufficient to be diagnostic in inflammatory neuritis.[3,4]

Protamide—an exclusive denatured colloidal enzyme preparation, virtually safe and painless—not foreign protein therapy. One ampul I.M. daily for 2 to 5 days usually relieves pain completely in patients treated early.

SUPPLIED: boxes of 10 ampuls (1.3 cc.). For detailed information, refer to PDR, page 731, or write to our Medical Department.

References: 1. Baker, A. G.: Penn. Med. J. 63:697 (May) 1960. 2. Smith, R. T.: New York Med. (Aug. 20) 1952, pp. 16-19. 3. Smith, R. T.: Med. Clin. N. Amer. (Mar.) 1957. 4. Lehrer, H. W.; Lehrer, H. G., and Lehrer, D. R.: Northw. Med. (Nov.) 1955. 5. Sforzolini, G. S.: Arch. Ophthal. 62:381 (Sept.) 1959.

Sherman Laboratories
Detroit 11, Michigan

in severe drug and food sensitivity... rapid relief and control of symptoms on short-term therapy with Decadron®

Brief treatment with DECADRON – orally or parenterally – can provide rapid and effective control of allergic emergencies and acute allergic disorders such as reactions to foods, drugs, plants, weeds, and animals. In 40 patients given Injection DECADRON Phosphate, "subjective improvement was often noticed within one hour and objective improvement recorded within 4 hours."[1] Therapeutic doses of steroids may help prevent recurrences of severe allergic states, without interfering with desensitization or other immunity procedures.[2]

Before prescribing or administering DECADRON, the physician should consult the detailed information on use accompanying the package or available on request.

References: 1. Grater, W. C.: Southern M. J. 53:1144, 1960. 2. Feinberg, S. M.: Med. Sci. 6: (No. 3) 181, 1959.

Supplied: As 0.75 mg. and 0.5 mg. scored, pentagon-shaped tablets in bottles of 100 and 1000. As Injection DECADRON Phosphate in 5 cc. vials, each cc. containing 4 mg. of dexamethasone 21-phosphate as the disodium salt; inactive ingredients: 8 mg. creatinine, 10 mg. sodium citrate; sodium hydroxide to pH 7.8, and water for injection q. s. 1 cc.; preservatives: 0.32 per cent sodium bisulfite and 0.5 per cent phenol. DECADRON is a trademark of Merck & Co., Inc.

MERCK SHARP & DOHME Division of Merck & Co., Inc., West Point, Pa.

Decadron®

Dexamethasone

TREATS MORE PATIENTS MORE EFFECTIVELY

DECADRON: Recommended dosage schedule in the treatment of drug and food sensitivity reactions

time	amount	administration
1st day	one to two cc. (4 to 8 mg.) Injection DECADRON Phosphate intramuscular	repeated as necessary (In substituting tablet therapy, give the first oral dose four or five hours before the final parenteral dose.)
2nd day	two 0.75 mg. Tablets DECADRON	b.i.d.
3rd day	two 0.75 mg. Tablets DECADRON	b.i.d.
4th day	one 0.75 mg. Tablet DECADRON	b.i.d.
5th day	one 0.75 mg. Tablet DECADRON	per day
6th day	one 0.75 mg. Tablet DECADRON	per day
7th day	RETURN VISIT	

Dosage and administration — Adults: Anadrol is administered orally. The usual adult dosage is 2.5 mg. three times daily. The majority of patients show a favorable result at 7.5 mg./day. However, because of its comparatively low degree of androgenicity, doses up to 15 mg./day may be employed. Results have been seen in 10 days, but optimal results are usually obtained in 4 to 6 weeks. Therapy may have to be continued to sustain improvement according to the needs of the individual patient.

Children: Adequate response has been observed in children up to the age of 12 years on dosage schedules of 2.5 mg./day and 5 mg./day. Children over 12 years of age may be given adult doses.

Precautions — Side effects with Anadrol have been minimal when administered in the recommended daily dosage range. In those few instances where masculinizing effects have been noted in adults or children, reversal has been accomplished after discontinuing therapy.

Although Anadrol exhibits only a slight androgenicity, it shares with all androgens a tendency to favor sodium retention. For this reason, the drug should be used with caution in patients with heart disease.

Administration to children should not be continued beyond a 30-day period because of increased sensitivity to masculinizing agents. If such masculinizing symptoms develop, this drug should be discontinued.

Contraindications — Anadrol is contraindicated in the presence of carcinoma of the prostate and, because of changes which have been observed in hepatic function on long-term use with Anadrol at higher doses, it should be used with caution in patients with known hepatic damage. It is also contraindicated in nephritis and nephrosis.

How supplied — Anadrol 2.5 mg. is supplied in bottles of 50 white, monogrammed tablets.

NEW PRODUCT BROCHURE AVAILABLE ON REQUEST.

anadrol™
oxymetholone, Syntex

ANABOLIC STEROID

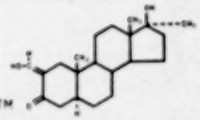
reverses the wasting process— provides solid weight gain

- Anadrol stimulates nitrogen retention with 4 times the potency of methyltestosterone.*
- Anadrol produces solid weight gain, not transient fluid retention.
- Anadrol is half as androgenic as methyltestosterone.*
- Anadrol (oxymetholone) is lower in virilization.*
- Anadrol, because of minimal side effects, is ideally suited for prolonged therapy.
- Anadrol is indicated for use in malnutrition and a wide range of catabolic disorders marked by a negative nitrogen balance.

*As determined in man

this preparation for use in:

Geriatric debilitation	Convalescence from infection
Chronic underweight	Gastrointestinal disease
Pre- and postoperative conditions	General catabolic conditions
Osteoporosis	Malnutrition



anadrol™

(2-hydroxymethylene-17α-methyl-17β-hydroxy-3-androstanone)

SYNTEX

51-GP-1A

not a general-purpose antibiotic

Albamycin is not a broad-spectrum antibiotic, recommended for routine infections. It is specific for staphylococci (including resistant strains), and its use alone should (with the exceptions listed below) be limited to those cases in which staph is known or strongly suspected to be the causative organism.

Albamycin*

Indications — Albamycin is indicated in the treatment of staphylococcic infections, particularly in patients sensitive to other antibiotics or in the infections in which the organism is resistant to other antibiotics and sensitive to Albamycin, and in urinary tract infections due to microorganisms resistant to other commonly employed antibacterial agents but sensitive to Albamycin — notably certain strains of Proteus.

Administration and Dosage — Capsules and Syrup: The recommended dosage in adults is 500 mg. every twelve hours or 250 mg. every six hours, continued for at least forty-eight hours after the temperature has returned to normal and all evidence of infection has disappeared. In severe or unusually resistant infections, 0.5 Gm. every six hours or 1 Gm. every twelve hours may be employed. The dose for children is 15 mg. per kilogram of body weight per day for moderately acute infections; this may be increased to 30 to 45 mg. per kilogram of body weight per day for severe infections. These doses may be administered on schedules similar to those for adults.

Parenteral: Intramuscularly—5 cc. of Albamycin solution may be used directly by slow injection deep into the gluteal muscle.

Intravenously — it is recommended that 5 cc. of Albamycin solution be diluted further with 250 to 1000 cc. of sterile injection solution of sodium chloride, Darrow's solution, or Ringer's solution and administered by intravenous infusion, or by diluting to a suitable quantity and administered by continuous drip infusion. **Do not use with dextrose solution.** When it is necessary to use a smaller volume intravenously, 5 cc. of Albamycin solution may be diluted to a minimum of 30 cc. with one of the above diluents and administered slowly over a period of five to ten minutes to avoid irritation of the vascular endothelium. The dosage for adults is 500 mg. Albamycin administered either intramuscularly or intravenously every twelve hours. For children with moderately acute infections, the dosage is 15 mg. per kilogram of body weight per day. The daily dosage should be administered in two divided doses at intervals of twelve hours. As soon as the patient's condition permits, parenteral Albamycin should be replaced with oral Albamycin therapy.

Side Effects — Albamycin is a substance of low toxicity but is capable of inducing urticaria and maculopapular dermatitis. Leukopenia, which was rapidly reversible, has been reported in approximately 1% of cases. All of these side effects disappear rapidly upon discontinuance of the drug. In a certain few patients, a yellow pigment has been found in the plasma. This pigment is a metabolic by-product of the drug which, however, may interfere with determination of bilirubin and icteric index. Its presence is not associated with abnormal liver function tests or liver enlargement.

Available — Albamycin, 500 mg., sterile, Mix-O-Vial.† Each Mix-O-Vial contains: 500 mg. Novobiocin (as novobiocin sodium), also 175 mg. Nicotinamide; 0.47 cc. N,N-Dimethylacetamide; 42.3 mg. Benzyl alcohol; 4.23 cc. water for injection. Albamycin Capsules. Each capsule contains: 250 mg. Novobiocin (as novobiocin sodium). Albamycin Syrup. 125 mg. per 5 cc. Each 5 cc. (one teaspoonful) contains: 125 mg. Novobiocin (as novobiocin calcium). Preserved with methylparaben, 0.075%, and propylparaben, 0.025%.

*Trademark, Reg. U. S. Pat. Off. — The Upjohn brand of crystalline novobiocin sodium. †Trademark, Reg. U. S. Pat. Off.

The Upjohn Company
Kalamazoo, Michigan

Upjohn

Schering

HOW RELA™ BREAKS THE PAIN-SPASM-PAIN CYCLE

ANALGESIC: RELA "...diminished the need for administration of analgesic drugs [aspirin, codeine, meperidine]."[1]

MOBILIZATION: RELA restores mobility by relieving pain, stiffness and spasm.

RELAXATION: RELA relaxes, eases acute muscle spasm and pain through its integrated analgesic-relaxant actions.

CLINICAL EFFECTIVENESS: "The effects of carisoprodol [RELA] were shown by relief of pain, and relief of localized muscle spasm...."[1]

RAPID RECOVERY: One fourth the recovery time—RELA treated group of 106 low-back patients averaged 11.5 days—control group, 41 days.[1]

RELA™ RELAXES, EASES ACUTE MUSCLE SPASM & PAIN

CARISOPRODOL 350 mg. TABLETS

Bibliography: 1. Kestler, O. C.: *J.A.M.A.* *171*:2039 (April 30) 1960.

Complete information on RELA including indications, dosage, side effects, and precautions is available to physicians on request.

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